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ERRATA

Page 5, line 16. For *Rochea* read *Kleinia*.

Page 23, third paragraph, third line. For "phellonic" read "phloionic,"
and for "considerable" read "small."

Page 136, bottom. For "S. H. W." read "S. M. W."

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EDITORIAL

THE past three years have been an anxious time for those financially responsible for the publication of periodical scientific literature, and the anxiety is not yet removed. The costs of production have increased more than threefold from the pre-war level, continental subscriptions have not been renewed owing to the unfavourable or even prohibitive rates of exchange, and many home subscribers have been compelled to cease their subscriptions owing to the increased cost of living. These difficulties have been partly met by various economies, by raising prices and by reducing the bulk of matter, but such measures have limits beyond which they cannot be carried without defeating themselves. No journal can be permanently successful which does not present a reasonable amount of varied and attractive matter at a price which its readers can afford.

The moment therefore seems opportune for an appeal to contributors to do their part in helping to carry out these objects, since it is to the interest of contributors and readers alike that a scientific journal should contain as much varied matter in as short a space as is practicable, and should have as large a circulation as possible.

The two points to which attention should particularly be directed are these:

1. Contributions should be arranged as logically and written as concisely as possible. A large proportion of the scientific papers that are published would gain substantially from rearrangement and condensation of the matter.

2. The copy should be very carefully revised before it is sent in. The author should make sure that it is in the exact form in which he wishes it to appear. A considerable amount of money is often wasted on corrections and alterations in proof which could perfectly well have been made in the copy.

It is proposed in future to send out first proofs in page form, and to charge to the author alterations exceeding 15 per cent. of the cost of composition.

The Editor confidently appeals to all contributors to bear these points in mind in preparing papers for publication.

Each contributor will in future be presented with 50 separate copies of his contribution free of charge. When the contribution occupies four pages or less the separate copies will have no covers.

THE BIOCHEMISTRY OF CARBOHYDRATE PRODUCTION IN THE HIGHER PLANTS FROM THE POINT OF VIEW OF SYSTEM- ATIC RELATIONSHIP

A CONTRIBUTION TO THE DISCUSSION ON BIOCHEMICAL CLASSIFICATION OF
PLANTS AT THE CARDIFF MEETING OF THE BRITISH ASSOCIATION

By F. F. BLACKMAN

IN surveying the Carbohydrate Economy of Flowering Plants as a whole, we note that there is a general uniformity, interspersed with some strikingly aberrant cases. These exceptional cases are not distributed indiscriminately, but tend to characterize groups of plants which are classed as related systematically, which we interpret in terms of common phyletic origin. The attempt of the present contribution is to review some of these biochemical diversities of carbohydrate production and see what they look like as steps toward a biochemical classification of plants, and how they rank with the morphological characters on which groups of flowering plants have been mostly drawn up.

Carbohydrate production can be analysed into three strata, representing progressive stages of what we may call the up-grade carbohydrate flux.

We have (1) the primary photo-reduction of carbonic acid involving light-energy and specific pigments: (2) the immediate appearance of sugars; which seems to be universal: (3) the subsequent appearance, though by no means universally, of complex polysaccharides, which are deposited in the chloroplasts. Similar bodies are also formed in the leucoplasts of storage organs from sugars arriving from the leaf by translocation.

These three stages represent increasing chemical condensation to larger and larger aggregates. The first stage, derived from reduction

of carbonic acid may be assumed to be, initially, a molecule with one carbon atom. Formaldehyde is the substance that satisfies all theoretical considerations, though its actual occurrence has not been proved. For the second stage we jump to sugars with 5 and 6 carbon atoms, pentoses and hexoses, while from the latter by doubling we get 12-carbon sugars, of which cane-sugar is the most obvious. For the third stage we have much condensed polysaccharides like starch, inulin, etc., in which the number of carbon atoms may run up to several hundreds.

Now as we are making bold to consider the biochemical vagaries of protoplasm with a critical detachment, we may ask why these stages occur and not others, why the plant revels in the hexoses, makes a certain play with pentoses but does next to no traffic in sugars of 3, 4, 7 or 8 carbon atoms—the trioses, tetroses, heptoses, and octoses, all of which are preparable in the laboratory. The hexose, glucose, is undoubtedly the key-sugar to carbon-metabolism whether we consider up-grade processes or down-grade ones like respiration and fermentation. Considered purely chemically, glucose is a molecule of remarkable properties, combining a certain stability in pure solution with an extraordinary potentiality of varied chemical change in presence of what the pure chemist might call impurities. Now biochemistry is not the chemistry of pure substances but the chemistry of impure substances, indeed of very impure substances. It is a mild suggestion that always in the cell, several dozen impurities are present with any one substance that we concentrate attention upon.

In considering the important *rôle* that various sugars play, the biologist sometimes drifts into the teleological way of thinking, and regards the cell as having selected these sugars as specially suitable for its purposes.

Let us turn then to the test-tube for illumination on this matter, and particularly to the wisdom drawn from the test-tube by Nef in his elaborate researches on what we may call the purely chemical metabolism of sugar molecules. Nef, at Chicago, up to his recent death, devoted some fifteen years to studying the spontaneous chemical changes undergone by sugars in the presence of impurities, such as salts and hydrates of sodium and other metals. He showed how every single sugar, be it of 1, 2, 3, 4, 5 or 6 carbon atoms, tends to pass over into all the others, so that an equilibrium mixture of more than a hundred derivatives may arise in the course of weeks

by spontaneous interactions. All this radiating flux represents the purely chemical background of possibilities out of which the cell forms its particular uni-directional stream-lines of metabolic flux. What we have to investigate in protoplasm is the mechanism that determines change in one direction rather than others, out of the chemically limited possibilities.

To return to the fact that hexoses and pentoses occupy the first and second place in carbohydrate metabolism to the exclusion of the adjoining classes of tetroses and heptoses. Nef has shown that this is inherent in the structure of the different sugar molecules themselves. When formaldehyde is condensed by lead hydrate *in vitro* it is found that the end-products are almost entirely derivatives of just these two classes of sugars. Lower sugar-classes have merely a transient existence as intermediate stages in the test-tube—as in the cell; and in neither does condensation go on to higher classes of sugars such as heptoses.

We get real light on the cell and its possibilities and limitations from his general conclusion that just those individual hexoses which are abundant in plants, are exactly the ones whose derivatives tend to accumulate in the test-tube, having a certain inherent degree of stability. Nef's picture leads us on further, to imagining traces of innumerable sugar derivatives and forerunners present in the cell, as transitional states. Research directed towards more complete identification of cell-activity with purely chemical activity *in vitro*, on these lines, is very difficult and hardly begun yet. Up to the present we have had to be content with identifying only those substances which occur in bulk in the cell.

Understanding now why hexoses are so abundant in carbohydrate economy, our attention may next be turned to pentoses.

The drift of recent opinion is towards believing that pentoses really play a more important part in the cell than has hitherto been recognized. Pentoses, and their particular polysaccharides, pentosans, are important constituents of the nucleus, of certain cell-walls, and of mucilage, but little general metabolic significance has been allowed them.

Now, in a particular type of plant, pentoses and pentosans are abundant. Spoehr in his work on the carbohydrate economy of Cacti has emphasized their importance in relation to plants that exhibit the type of structure and physiology that is known as 'succulent.' Recently a remarkable pronouncement has been made on the real factor which determines the succulent habit, which habit

had before been attributed to the water-relations of succulent plants to the arid soils in which they grew. In this paper on 'The Basis of Succulence' three Americans, MacDougal, Richards and Spoehr, who have worked more or less independently on succulents, have come to the agreed conclusion that succulence is due to a "metabolic complex favourable to pentose formation." Pentoses lead on, by condensation, to pentosan-mucilage with special water-holding properties, and to other characteristics so marked in succulents.

We may accept it then that here we have a deep-seated aberration of the ordinary carbohydrate flux tending more to the production of pentoses than usual. Here is one of those biochemical variations that we spoke of in opening, and it becomes of interest to note the distribution of succulence among flowering plants. Certain families of plants are characteristically succulent, such as Cactaceae and Crassulaceae, but we find succulent genera and groups of genera here and there among other families. *Rochea*, among the Compositae is a well-known example of an isolated succulent genus, but it possesses all the correlated physiological and anatomical properties that are found in the typical succulent families.

It would seem that we must assume that the mutation in protoplasmic constitution, which determines this direction of carbohydrate-flux, has occurred a number of times independently and that in some cases this has been followed by evolution of a whole group of allied genera and species, retaining the character, while in other cases only a single isolated form perpetuates the mutation.

After this example let us turn to a biochemical point in which there is great variation through the range of flowering plants. This concerns the transition from the second stage, that of sugars, to the third stage, that of polysaccharides. It is well known that a number of plants form starch freely in their chloroplasts, as a so-called 'temporary reserve' of the carbohydrate produced by photosynthesis, while many plants, on the other hand, form little or none. Mayer, in 1885, investigated all the plants in a botanic garden from this point of view and he found that this characteristic mostly holds true throughout a family of plants. He grouped the families examined into five classes, running from those that form starch very richly—Class I—to those that form none at all—Class V. Among Dicotyledonous families, most come in Class II, Solanaceae and Papilionaceae being alone in Class I, while Class V is represented only by Gentianaceae. When we come to Monocotyledonous families, it is

striking to find no family worthy to come in Class I or Class II, while most of them come at the tail in Classes IV and V. Here we seem to have a definite shifting of a biochemical character, possibly early in the separation of the two morphological types. Isolated exceptions occur to this general relation, as for example, *Hydrocharis* among the Monocotyledons which condenses its sugar to starch in great abundance. Both types of leaf, the starch-leaf and the sugar-leaf, seem to be quite adequate, physiologically, and neither suggests any real biological advantage over the other.

This sugar-leaf and starch-leaf distinction can, however, be analysed a stage deeper, so that we believe we know what it turns upon. It is not due to any lack, in Monocotyledons, of the agent which determines the condensation of sugar to starch, but to the high critical concentration of sugar that is needed to start starch-formation. If sugar can be got into the cells in sufficient concentration, then starch is visibly formed in leaves of the sugar-leaf type also. This can be done quite simply by floating cut pieces of leaf upon strong enough sugar solution in the dark. In 1898 Winkler showed that with ordinary starch-leaves, 0.2 to 0.5 per cent. sugar is the critical concentration. The moss *Mnium* gives the lowest value of 0.05 per cent. For sugar-leaves we have values like 15 per cent., while the leaves of the sugar-cane are found to require 18 per cent. sugar. Only one plant has such a high value that it has resisted all attempts to force it into starch-formation, and that is the Onion, which can be got to store nothing but sugar however it is treated.

This 'critical concentration' theory is supported by all workers on the subject. It indicates that there is not a simple equilibrium-relation between starch and sugar; and that, as Lundegardh has urged, starch-formation is a complex happening still requiring a good deal of quantitative investigation. One of the fascinating points of departure from simple chemical expectation is, that cane-sugar is a better sugar for artificial starch-formation than either maltose or glucose.

We judge then that Monocotyledons are characterized by a high critical sugar-concentration generally, while Dicotyledons show a low one. This distinction seems to be merely one of degree and not of kind, so that it is hard to say to what sharply defined protoplasmic character it may be attributed. The distribution of this character over families is quite like that of succulence, showing here a wide sweep over a large number of related genera and there an isolated example.

These biochemical characteristics, then, in the matter of their scatter, remind one of those morphological characteristics which are of secondary or tertiary importance rather than of the fundamental distinctions which hold without exceptions for large aggregates of families.

For a last point we may take up the fundamental problem of whether a biochemical classification of plants is conceivably attainable, given enormously increased knowledge. Assuming that all morphological as well as all metabolic features of plants must be the outcome of definable attributes of protoplasm, it seems clear that even if we could state these, we should still have the difficulty of balancing up conflicting affinities to decide phylogenetic relationship and that, as now, everything would turn upon consensus of evidence, before the biochemist could arrange his metabolic variants in families, genera, species and forms.

We may well assume that the protoplasm of every form differs from that of every other in some particulars and that these differences, at the minimum, might be those of a set of systems composed of optical isomers. The richness of protoplasmic systems in proteins and carbohydrates would make far more stereo-isomeric variants possible than there are different living species and forms. On this basis plants would be classified by the configuration of their protoplasmic atomic groupings in space, instead of by the configuration of their flowers and allied features. Ultramicroscopic form and morphology would replace macroscopic.

A thorough-going attempt has been made recently to explore plant-protoplasmic activity to see whether such a conception of innumerable different stereo-isomeric protoplasms corresponds to anything in the actual facts of biochemistry. This laborious work has been undertaken by Reichert and the Carnegie Institution.

After Reichert had shown that the haemoglobins of all animal-genera examined could be differentiated by crystallographic and other characters, so establishing a biochemical specificity of animal organisms, he decided to extend the same sort of enquiry into the plant kingdom.

The substance selected was starch. His underlying conception was that branches of the protoplasm in amyloplasts responsible for the condensation of starch would probably possess slight stereo-chemical differences from species to species and that this must determine corresponding differences in the arrangement in space of

the condensed hexose units. The lowest molecular weight for starch is 15,000, which indicates the condensation together of at least 90 hexose units and so suggests many more possible stereo-isomers than there are starch-bearing plants.

Reichert's first investigation, published in 1913, was directed to enquiring if the starch-grains of all species of plants are demonstrably different. For the 300 species examined the answer is in the affirmative. A few weeks ago there reached this country a second publication containing a minute study of the starch-grains of parents and crosses. These two investigations together fill four huge quarto volumes for which the Carnegie Institution has subsidized the publication of 800 photo-micrographs of starch-grains, 1100 graphic charts of their reactions, as well as some 1700 pages of text.

For each plant, the isolated starch-grains are examined for some twelve characteristics; four points of visible form, two of appearance in polarized light, two of staining with aniline dyes, several of reactions with iodine and other agents and finally the solution-temperature or rate at which the grains lose their solid form when heated with water, which temperature ranges from 49° to 90° Centigrade. No two of these characteristics seem to be closely correlated and Reichert treats them all as 'unit characters.'

Graphic charts are constructed by giving a numerical value to the degree of reaction of a given starch to each of these agents, so that the eye can take in, as a whole, all the different behaviours of each starch in the form of a curve. No great accuracy is claimed for the detailed values, as the whole work is entitled a preliminary exploration. The results certainly support the thesis that no two species or cultural forms have quite identical starches, and that in crosses the starches of the cross show a varying behaviour, but incline more towards the characters of the seed-parent.

From his review of genera and species, the author concludes that nearly always the starches of species of one genus resemble each other more than they resemble species of other genera. Genera, too, often show similarity within one family. Indeed, it is interesting to find how closely, on the whole, the starch-characters accord with the subdivisions of classification accepted for flowering plants. Starch-evidence has definite contributions to make to the doubtful relationships of certain genera, and indicates that some families are homogeneous and others heterogeneous.

No individual details are given as to the part of the plant the starch-grains were taken from, but the general statement is made

that, in all cases, it came from organs subserving vegetative reproduction. We should be glad to learn the characters of the starch-grains formed in the leaves of some of these plants for comparison. If all plastids arise from a common origin by division we should expect definite relations, on the present hypothesis, between the starch products of chloroplast and of leucoplast. As Reichert says, an enormous amount remains to be done, but the whole situation is full of suggestion and interest. There is however, as yet, no indication that the different starches are merely stereo-isomers. The researches of Max Samec show what a complex colloid one has in natural starch.

In conclusion, we may recall how differently the matter stands with the pigments of the chloroplasts from species to species. It is true that Étard in 1906 asserted that there were an enormously large number of different chlorophylls in different plants, but all this has disappeared before Willstätter's demonstration of the extraordinary uniformity of the chlorophyll pigments throughout the higher plants. Influenced by this identity of pigment, we imagine identity in the simple first product of photo-reduction of carbonic acid in all cases. From this initiation the up-grade carbohydrate-flux proceeds; the first stage of arrest we may attribute to the properties of sugar molecules themselves, while the final products of condensation are determined by the minute chemical configuration of the protoplasmic agents in contact with which this condensation proceeds.

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HETEROTHALLISM AND SIMILAR
PHENOMENA

By E. M. CUTTING

IN 1906 *The New Phytologist* published a critical *résumé* of work (5) by A. F. Blakeslee on the presence of two kinds of mycelia in certain forms of Mucorine fungi. Since that date there have been accumulating a certain number of new facts bearing on the problem presented by the communication mentioned above. It is the purpose of this note to review shortly these disconnected observations and to attempt to indicate certain points of contact between them.

Of these, one of the most interesting is contained in a preliminary note by Burgeff (12), followed by a fuller illustrated account, in *Flora*, 1914 (13). This author worked mostly on *Phycomyces nitens*, a species that had previously been investigated by Blakeslee, who found that it possessed + and - forms, or in other words, was heterothallic. By means of a very delicate and difficult technique "grafting" was accomplished between a + and a - form of mycelium. The mycelial outgrowths resulting from this operation formed sporangia, which contained three kinds of spores, those which gave rise to + mycelia, those that formed -, and those that formed neutral hyphæ. These results were regarded as in keeping with the view, which is the one held by Burgeff himself, that the "plusness" or "minusness" resides in the nuclei themselves. The spores of *Phycomyces*, it will be remembered, are multinucleate and it is held that the neutral strain is formed when - and + nuclei are present in about equal numbers.

Blakeslee (6) has stated that in the heterothallic Mucors, so far as his investigations carried him, the zygosporangia germinated to form a sporangium which contains either + or - spores, so that a segregation occurs before the formation of sporangial spores. There was no evidence to show the method of this segregation. In *Phycomyces nitens*, however, the germ-mycelium was neutral and bore sporangia containing spores which gave rise to plus, minus and neutral mycelia; and the neutral mycelium had the power of forming abortive or imperfect zygosporangia with either - or + mycelia.

The experimental results of Burgeff fill in a gap in the observations given above. We have here, seemingly, a vegetative segregation caused by a differential nuclear distribution.

Blakeslee himself recorded in 1915 the formation of so-called imperfect hybrids, *i.e.* the partial union of reproductive bodies, when he grew dioecious species of *Zygorhynchus* between a + and a - *Mucor* (9, 10). The plus form showed imperfect fusions with the larger gamete of the *Zygorhynchus*, and the minus form with the smaller. On these grounds the plus race is regarded as the male-bearing thallus and the minus race as the female. The same opinion had already been expressed by Blakeslee from other considerations. It is a debated point whether these gametes of *Zygorhynchus* can be regarded as male and female in the usual sense, for it has been claimed that the contents of the larger cell are emptied into the smaller cell. Gruber (25) regards the larger as male and compares the process with that taking place in the Oomycetes. These results obtained by Gruber have been criticised by Moreau (34) who is of the opinion that the process in *Zygorhynchus* falls into line with the rest of the Mucorines, and that Gruber has erred through misinterpreting the position of the suspensor.

A point of further interest arises when it is found that G. F. Atkinson (1) holds that the larger cell in *Zygorhynchus*, supposed by him to be female, is provided with a trichogyne by means of which connection is made with the male organ, the smaller gamete. Atkinson also holds that *Zygorhynchus* is a lowly form of Ascomycete and a connecting bridge between this group and the Zygomycetes. It is not our purpose here to discuss the origin of any fungal group, but if *Zygorhynchus* be regarded as an Ascomycete the partial fusions with heterothallic Mucorines are even more difficult to explain.

A similar cross-septum has been reported in *Zygorhynchus* by Gruber (25), who also comments on its temporary nature but attempts no morphological interpretation. Further cytological observations should clear up these difficult points.

Burger (14) has recently been working on strains of *Cunninghamella*. He was unable either to get his results to fit those of Blakeslee or to suggest an alternative theory. I have specially mentioned this work for it is probably through an examination of such aberrant cases that further light will be thrown on the whole problem. Careful cultural experiments with species having uninucleate spores (and occasional multinucleate ones), and a comparison with multinucleated spore species, especially with regard to their heterothallism and heterogamy, would probably be helpful also.

No attempt will be made here to deal with the question of the methods of reproduction amongst the Myxomycetes. The Oomycetes,

however, which we will now consider, have been regarded by Clinton as showing indications of the presence, amongst them, of physiological strains(16, 17).

This observer found in cultures of different species of *Phytophthora* an abundant formation of oospores, interpreted by him as hybrids, at the place of intersection of the hyphæ. Murphy, working on *Phytophthora erythroseptica*, found a similar formation of oospores where neighbouring growths of hyphæ met, and, at first, was inclined to think that he was dealing with a heterothallic form(35). Single hyphal cultures, however, were capable of fruiting alone when any check was made to the further growth of the fungus, as by cutting out a piece of the medium in the track of growth of the hyphæ, oospore-formation immediately began. Murphy holds, therefore, that the phenomena observed by him was due to a nutritional check to the vegetative growth, initiating the sexual reproductive phase, and he thinks that Clinton's results, mentioned above, can be explained in a similar way.

Such interruptions do not necessarily induce an increased formation of reproductive bodies amongst the fungi.

Fitzpatrick has been investigating the origin of the binucleate condition in *Eocronartium muscicola*, a member of the Auriculariales; the germination of the uninucleate spores did not offer any solution, nor did an examination of its mycelium in one of the host plants give any further insight, as all the cells were binucleate(22, 23). There were no clamp-connections, however, so that it was not possible for migrations to take place there similar to those observed by Kniep in *Corticium varians* Kniep and *C. serum* Pers. The cross-walls of *Eocronartium* are provided with metachromatic granules and in the large number of fungi, in which such granules are found so associated, it is also usual to find a pore in the cross-wall. It is possible that migrations in *Eocronartium* and similar Basidiomycetes may take place through such pores, as such migrations have been reported amongst the Ascomycetes(18, 26, 43). The only function that was widely accepted for the clamp-connections before this work of Kniep's was that they facilitated the nutritive relations of the component cells of the hyphæ.

Kniep, it should be mentioned, has also found that a hypha after forming a basidium may go on growing and forming other basidia, in a manner which strikingly suggests a comparison with the account of the ascus-formation in *Pyronema*, as given by Claüsssen; and, in fact, Kniep does hold that the ascus and basidium are homologous

structures. The whole series of arguments may be profitably studied in his papers (29, 30, 31).

The opinion of Maire and of Miss Nichols (36), it may be remembered, is that, in the forms investigated by them, the binucleate conditions arose by simple division from the uninucleate condition. This may well be in some forms. Kniep, some few years ago, showed that in *Armillaria mellea*, basidia can arise on a uninucleate mycelium, so that here the conjugate stage is entirely left out (28). Miss Bensaude (3, 4), in a preliminary statement has briefly recorded her observations on an unnamed species of *Coprinus*, and her full thesis has since appeared: in this she describes the development of *Armillaria mucida*, *Tricholoma nudum*, and especially of *Coprinus finetarius*. The hyphæ of the mycelium formed from a single spore exhibited anastomoses, but no clamp-connections, and never gave rise to the binucleate condition nor did they form fruit bodies. With mixed sowings, however, migrations take place after cell-fusions, the fusions being between mycelium and oidium or another mycelium; and in this manner the binucleate condition is brought about. Miss Bensaude is therefore of the opinion that the species of *Coprinus* investigated by her is heterothallic like some of the Mucors. Brefeld was able to grow fruit bodies, of another species of *Coprinus*, from one spore, so that Miss Bensaude does not expect that the method discovered by her will be found to be general among the Higher Basidiomycetes. The way in which the nuclear association is brought about reminds one of the similar process in *Humaria rutilans* (24)—except that here nuclear fusions immediately follow the binucleate condition—and more especially of the state of affairs in the Uredineæ (37) and in the Hemibasidii (2), in some of which the conjugate condition continues for a considerable period. No phenomena suggesting the occurrence of heterothallism have been met with in the Uredineæ, Bunts or Smuts.

In one genus of the Ascomycetes a phenomenon that seems allied to heterothallism has been found by Edgerton (20, 21). Here what had been formerly regarded as two different species of *Glomerella* are found to produce a larger number of fruits than are formed by either mycelium alone and under conditions that do not seem to point to hybridization.

This fungus forms but few ascocarps in the minus mycelium and the asci are small; a considerable number of asci are formed on the plus mycelium; at the junction of the two kinds of mycelium the perithecia form abundantly and contain large, well-developed asci,

the spores of which give rise some to + and some to - mycelia. This causes Edgerton to regard the formation of perithecia at the junction of + and - mycelia as being consequent on a sexual act. We are, however, quite ignorant of the method of origin and development of the ascocarp in this form and it is to be hoped that some information on this subject will soon be forthcoming. Shear and Miss Wood⁽³⁹⁾ have investigated this *Glomerella* and are of the opinion that hybrids are formed. Edgerton, as already mentioned, finds himself unable to accept this view. A side issue, but an important one, is to be found in the fact that this fungus is a parasite and the presence of both strains in a district would probably cause a larger spore-formation and so indirectly lead to greater destruction than if one form only were present. It will be seen that, whatever the cytological examination of these forms may result in, the case of *Glomerella* differs from all those so far described in that both its strains can form ascus-producing fruits; the result of the presence of both being that a greater number of ascocarps than usual are produced.

A case which, in a way, seems to resemble this, has lately been shortly described by Taubenhaus⁽⁴²⁾. In *Sclerotium rolfsii* there were indications of the formation of abundant sclerotia when certain kinds of mycelia met, these sclerotia differed in no ascertainable way from the sclerotia formed on any other mycelium and we might regard the phenomena here exhibited as being due to an interruption of vegetative growth, as in the case of oospores in *Phytophthora erythro-septica*, described by Murphy⁽³⁵⁾, were it not that Taubenhaus does not get the phenomena to take place at the point of contact of any kind of mycelial growth. This worker was unfortunate enough to have his cultures destroyed, by mistake, when he was away at war-work, and was unable to follow the matter any further on his return. It will be difficult to get an explanation of this behaviour, *i.e.* the abundant formation of, presumably, vegetative resting bodies, which will also include the other cases mentioned above.

In various other Ascomycetes, *e.g.* in *Diaporthe Batatatis*⁽²⁷⁾ and in *Gibberella Saubinetii* (Dickson and Johann⁽¹⁹⁾) various strains have been recorded, and in the Basidiomycetes considerable difficulties have been encountered in bringing them into fruiting stages. In these cases, where external conditions are not the factors concerned, as well as in the Fungi imperfecti, the observations of Miss Bensaude and of Edgerton point to a new manner of tackling the problem of obtaining the higher fructifications.

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POSTSCRIPT

Since the above was written an important summary of non-cytological work on the sex of the Mucorineæ by Namyslowski has appeared in the *Revue Générale de Botanique* (May 1920). Amongst other interesting facts, our attention is drawn to the opinion, which seems to be growing prevalent, that a series of transitions exist between homothallic and heterothallic forms, and that external conditions greatly modify the character of the method of reproduction. Burger's results on *Cunninghamella* and the behaviour, reported by Edgerton, of the ascomycete *Glomerella*, are in keeping with this view, but not with that originally put forward by Blakeslee. It will be evident, moreover, from a general knowledge of plant physiology that the phenomena of "Zygotactism" and of "Haptomorphism" may be expected to vary much with external conditions, and the latter may indeed prove to be replaceable by some other stimulus.

SUBERIN AND CUTIN

By J. H. PRIESTLEY

INTRODUCTION

DURING an investigation of the physiological rôle of the endodermis it became necessary to describe the composition of the radial and transverse walls of this tissue, which from an early stage in their differentiation appear relatively impermeable to water. The impermeability to water of a plant membrane seems usually to have been ascribed to the presence within it of suberin or cutin, but in using these terms the writer was very conscious of the lack of precision with which he employed them. Reference to current English text-books revealed a similar vagueness even in the most recent monographs on plant biochemistry. However, a search through the literature showed, that during the last thirty years a great deal of light had been thrown upon the subject by the research of different continental investigators. The work seems so important and so neglected in this country that it is proposed to give a brief account of it in this paper.

Although this is a review of earlier work upon the subject, no lengthy bibliographical record is attempted, but reference to the papers cited will enable any reader to obtain the nucleus of an excellent bibliography. On the biochemical side Czapek's book(4), p. 695, contains a very valuable chronological summary. Here it is only possible to emphasise the most salient points in the development of our knowledge of the subject.

Most of our knowledge refers to suberin which is available for study in convenient form in the shape of bottle cork, the but little altered periderm of *Quercus suber*. Cutin receives elucidation usually indirectly as result of the work done upon suberin. So far, those substances are rather distinguished by their position in the plant, than by their properties, suberin being formed in the periderm, cutin in the outermost layer of many epidermal walls which are thus provided with a cuticle.

It will be seen from the account which follows that suberin and cutin, although still far from completely known, are names for aggregates of substance occurring in or on plant membranes and differing in their composition as well as in their distribution in the plant. The component substances of these aggregates have to some extent been

isolated and described and a suggestion can be made as to the form in which they are present within the wall. Furthermore, the ground seems clear for future work which may be expected to elucidate the nature and origin of the substances in the cell from which suberin and cutin are first formed, and also the conditions under which proceeds the transformation of these substances into suberin and cutin.

The following account is written with the more confidence because practically all observations which the present writer regards as fundamental have been repeated in his laboratory by different students. In this connection he has particularly to thank Miss M. Hind, B.Sc., Miss Edith North, B.Sc., Miss R. Rea, B.Sc., and Miss B. Lee.

TOWARDS A DEFINITION OF SUBERIN—A COMPARISON WITH LIGNIN

The term suberin is due to Chevreul(2) and was given by him to a substance insoluble in water and alcohol and constituting seventy per cent. of the substance of bottle cork. This substance he regarded as responsible for the special properties of cork, impermeability to water, general insolubility and great resistance to acids, etc.

"Suberin" starts in the literature then as a substance generally responsible for the peculiar properties of cork, and it appears to have gained little in clearness in English botanical literature until the present time. The reason for this is easily seen. Most botanical investigations have followed the micro-chemical method in investigating cork. Naturally it has not been possible to get much further than that the substance which *appears* to give cork tissue or periderm its peculiar properties, is concentrated in a special lamella constantly present within the cell wall of the cork cell. To this substance or mixture of substances the name of suberin has been given by common consent.

The first full and clear description of the substance or substances in the lamella we owe to von Höhnelt(9) whose work remains a landmark in the study of the subject, and it will be useful to cite briefly the characteristics of suberin as recognised in von Höhnelt's monograph.

One difficulty has always been to distinguish suberin from lignin, as both lignified and suberised tissues are left behind by treatment with strong acids or cellulose solvents. Von Höhnelt points out that lignin possesses many more characteristic colour reactions, a statement which is even more true to-day when beside those with phenol acid salts, many more general colour reactions for lignin are known. (See Czapek(4), p. 689.)

Von Höhnel attached most importance to the reaction of suberin with oxidising agents such as nitric acid or Schulze's macerating solution (potassium chlorate in nitric acid), a reaction which he describes as the cerin or cerinic acid reaction because the suberin is completely oxidised to a waxy substance which he regards as an oxidation product of suberin and terms cerinic acid; lignin gives no such oxidation product.

For micro-chemical purposes the sections are placed in Schulze's macerating solution and warmed gently under the coverglass, the lignified walls are gradually destroyed, the walls becoming more and more transparent and behaving like pure cellulose. Suberised (or cutinised) walls are much more resistant. They remain opaque and dark in the cold reagent and sharply contrasted therefore with all other walls; on warming an ebullition of gas is seen from the cork walls, but they swell very little until at a stage in the process of warming they collapse, the outlines becoming very wavy and swollen, the contents of the membrane largely melting up into oily masses which finally become spherical and globular and solidify on cooling. These globules are said to consist of cerinic acid, a substance first obtained by a similar process by Doepping⁽⁵⁾. Doepping gave it the name, under the impression it had arisen by the oxidation of cerin, a substance found to a small extent in cork. Von Höhnel retains the name whilst giving very sound ideas for regarding it as an oxidation product of suberin.

Another reaction of great importance in the identification of suberin, as was also emphasised by von Höhnel, is its behaviour with caustic alkali. Both lignin and suberin dissolve in concentrated caustic potash on warming, but von Höhnel points out that the suberin containing wall is usually thrown into more marked disorganisation by the removal of the suberin, which gradually disappears from the wall on heating, usually going into solution with a strong yellow colour. The lignin dissolves out in a very different way, leaving the wall little altered by its departure; in the solution of lignin a marked yellow coloration is often observed so that too much importance cannot be attached to this colour change.

Anticipating later work this distinction may be emphasised. In the lignified membrane lignin and cellulose seem to be present in an intimate physical mixture¹, and after solution of the lignin the outline of the wall remains practically unchanged. But in the cork wall

¹ The reader is referred for recent evidence on this point to the very interesting paper by Robinson (13).

the suberin seems to form an intermediate median lamella *unmixed with cellulose*, and its solution is accompanied by a distortion of the inner cellulose wall which is often left free in the interior of the cell.

In stating this we are travelling beyond von Höhnelt who considered that when the suberin had been removed from the lamella a basis was left behind consisting of cellulose. His main argument for this belief is that after and during the treatment with potash the suberin containing lamella gave a violet or reddish-violet reaction with iodine or sulphuric acid or with chlor-zinc-iodine, a reaction that he considered due to the cellulose present when it is no longer masked with suberin.

We owe to von Höhnelt the conception that the normal cork wall consists of three layers—the outermost or the middle lamella, a median one or the suberin lamella, and an inner one of cellulose. All subsequent investigators have confirmed this conclusion, but one of the most careful of them, van Wisselingh(15), has modified von Höhnelt's description in one important particular. Von Höhnelt described the median lamella as suberin on a basis of cellulose, van Wisselingh describes it as suberin without admixture of cellulose. Before describing van Wisselingh's work, however, it will be desirable to describe some macro-chemical observations of Eugène Gilson(8). Van Wisselingh's observations, made with great care and precision, are entirely micro-chemical in nature. They are published in a long series of papers which were appearing during the time that Gilson's work was published.

Gilson's work, dealing with appreciable quantities of material by the normal manipulations of organic chemistry, threw great light on the nature of suberin and incidentally enabled van Wisselingh to assess the critical value of his own micro-chemical observations with much more confidence.

THE MACRO-CHEMICAL INVESTIGATION OF SUBERIN

Gilson's work is based upon the solution of suberin on warming with potash. Recognising that the process was probably accompanied by the formation of soluble potassium salts of organic acids as in the saponification of an ester or true fat with alkali, he proceeded, by the customary methods of organic chemistry, to attempt the isolation of any organic acids and alcohols that might be present in solution.

As suberin seemed to dissolve more completely in an alcoholic solution of potash, he carried out his original saponification of powdered bottle cork with three per cent. alcoholic potash.

His original paper must be consulted for details of his methods, but the results may be summarised as follows:

He established the presence of several organic acids, which he termed as a class the *suberogenic acids*. Some of these acids, notably *phellonic* acid and *phloionic* acid, he obtained pure and crystalline. He found traces only of one higher alcohol, glycerine, and gave reason for thinking that the suberogenic acids were present in suberin in other forms than that of glycerides.

Gilson gives full details of his manipulatory methods, which have been followed throughout in a repetition of the work in the laboratory by several workers. His statements have all been confirmed even as to approximate yield obtained, save that so far we have failed to isolate sufficient quantities of glycerine to obtain the crystalline glycerine tribenzoate with benzoyl chloride and thus place beyond doubt the production of glycerine as a result of saponification.

In any case, Gilson concluded from the small quantities of glycerine obtained on saponification, and from the insolubility of the original suberin in normal fatty solvents, that suberin could not be regarded as a typical fat.

Kügler⁽¹¹⁾ had previously shown that some 12 per cent. of solid matter could be extracted from cork by boiling chloroform, of which some 2.9 per cent. was the crystalline substance cerin previously referred to (p. 19). The rest of the substance extracted proved to be amorphous and Kügler considered it to be suberin. Explaining his inability to extract the rest of the suberin by assuming that it was protected from the action of the fatty solvent by the molecules of cellulose which enveloped it, and assuming solubility of suberin in fatty solvents, Kügler decided that it was to be regarded as a true fat.

Although suberin is known to stain with fatty stains—notably Sudan III (see Kroemer⁽¹⁰⁾ for an elaboration of this staining method) and Scarlet Red (Scharlach R)—Gilson's conclusion above seems soundly based and suberin cannot be regarded as a true fat¹. There remain two outstanding questions, in what form are the suberogenic acids present in suberin and do they unite in the formation of a single definite substance suberin or is suberin an aggregate formed from varying quantities of these suberogenic acids?

As already stated Gilson obtained two of these acids crystalline

¹ Schmidt (14) has confirmed the presence of a small proportion of glycerides in suberin and suggests that the suberogenic acids originally reach the lamella in this form, subsequently decomposing and releasing the suberogenic acids.

and pure, viz. phellonic acid M.P. 95–96° C., percentage composition suggesting the formula $C_{22}H_{43}O_3$, and phloionic acid, crystallising in fine white needles, M.P. 120–121° C., percentage composition varying with prolonged drying from $C_{11}H_{21}O_4$ to $C_{22}H_{40}O_7$.

Another amorphous substance, semi-liquid, which Gilson terms suberinic acid, was obtained in relatively large quantities and, if it can be regarded as pure, its composition would agree with the formula $C_{17}H_{30}O_3$.

Of these suberogenic acids, phellonic was most completely studied, being obtained relatively easily in considerable quantities in the pure state, but all three acids possess one very important property. Both the acid and some of the salts, on heating for some time in sealed tubes, tend to go over into other forms, possibly anhydride or condensation products, which differ materially from the original suberogenic acid. The latter is soluble in the usual fatty solvents, at any rate on warming, the anhydrides are quite insoluble; the acid or some of its salts may be soluble or at least have a tendency to swell in water, whilst the new product is quite unaffected by the presence of water.

The significance of these facts for the formation of suberin is obvious. An experiment which has been carried out successfully in the laboratory gives an excellent demonstration of the possible significance of the suberogenic acids in the formation of impermeable membranes.

An ordinary Soxhlet thimble, as used in fat extractions, consists of a fat free cellulose preparation, readily permeable to water. One of these thimbles was taken and impregnated with a concentrated solution of potassium phellonate in hot chloroform. The thimble was then dried until all the chloroform had evaporated and then sealed up in a wide glass tube after partial exhaustion by a Geryk air-pump.

This sealed tube was then heated to about 180° C. on successive days for a total of about twenty-four hours in all. The phellonate in the thimble partly volatilised on to the walls of the tube but enough was left to impregnate the thimble thoroughly with the resulting anhydride or condensation product.

After heating, the potassium phellonate could no longer be removed from the thimble by the action of boiling fatty solvents and was obviously altered in its nature. This new substance rendered the Soxhlet thimble completely impermeable to water, the outer surface remaining quite dry to the touch when the thimble was filled to the brim with water and left for several hours.

It thus appears that it is possible to prepare artificially condensation products of these suberogenic acids and their salts which have the reactions that have been cited as characteristic of suberin; and that these acids can be obtained from suberin by saponification. The conclusion is then rendered probable that suberin consists largely of anhydrides of these acids, possibly together with a small proportion of the acids combined with glycerine as glycerides or true fats.

Von Höhnelt, and Fremy⁽⁶⁾ before him, spoke of suberin and cutin as definite individual substances, but it is obvious in the light of Gilson's work that they are more probably aggregates or mixtures differing in their composition with variations in the original proportions of the suberogenic acids from which they are formed.

This is indeed the case, for Gilson himself showed that the cork of *Ulmus "suberosa"* differed in its constituent suberogenic acids from that of *Quercus suber*, phellonic acid, present in considerable quantities in the cork of the latter being quite absent from that of the former.

Possibly these suberogenic acids may have a practical future before them in water-proofing cellulose fabrics (see Cross and Bevan⁽³⁾, *loc. cit.* p. 235). Certainly they exist in nature in sufficient quantities if they are required; the Eriophorum peat deposits of Yorkshire consist largely of the preserved cuticle, endodermal and corky tissues of Eriophorum and some preliminary experiments by Miss Hind show that large quantities of these suberogenic acids can be readily obtained from this peat by saponification.

MICRO-CHEMICAL INVESTIGATIONS—NO CELLULOSE BASIS TO SUBERIN OR CUTIN LAMELLA

For the clear recognition of the difference between cutin and suberin, and between the suberin of different species, we must turn to van Wisselingh's papers⁽¹⁵⁻¹⁸⁾.

Van Wisselingh's methods need studying in the original papers, and show how much insight into the ultimate structure of the membrane can be obtained in spite of the limitations of micro-chemical technique. The basis of this method was a study of the progressive decomposition or melting of the suberin lamella under four different methods of treatment: (1) heating in pure glycerine over a range of temperature, the highest well above 300° C., (2) heating in glycerine after previous treatment in concentrated potash (50 per cent.), (3) heating over the same range of temperature in glycerine containing 10 per cent. potash, (4) warming in 10 per cent. alcoholic potash.

Van Wisselingh tried all these methods on the tissues of certain selected plants and compared carefully the widely differing results he obtained. It would take too long to show how, by careful comparison of one series of results with another, he gradually arrives at conclusions as to the nature of the suberin or cutin in the particular plants under investigation, conclusions which seem to the present writer very critically established.

We must confine ourselves to two general conclusions of van Wisselingh's, first that the suberin lamella has no basis of cellulose, secondly that the suberin and cutin are not definite substances but varying aggregates.

Van Wisselingh, repeating von Höhnel's observation that the suberin lamella during treatment with potash began to give staining reaction with iodine and sulphuric acid or with chlor-zinc-iodine, had already published⁽¹⁶⁾ his reasons for doubting whether this reaction could be due to cellulose. Gilson later published his paper, describing the isolation of phellonic acid (see p. 21) and showed that both phellonic acid and its salt gave red to red-violet coloration with the iodine reagents used and suggested that von Höhnel had been misled by the production of phellonic acid during the saponification of the suberin by potash. Van Wisselingh then returned to the subject⁽¹⁷⁾; he showed that the coloration with iodine during treatment of the lamella with potash is a transient phenomenon, whilst cellulose would remain during this treatment; he further showed that after complete removal of the suberin, either by heating to 300° C. in glycerine or by saponification in 10 per cent. alcoholic potash or 10 per cent. glycerine potash, the suberin lamella gives no trace whatever of cellulose, and he gave further reasons for thinking that the reaction occasionally obtained is due either to phellonic acid or its salts or possibly in some cases to yet another suberin constituent.

Similarly, van Wisselingh gave reasons for considering that in the case of the cuticle, where we have present a layer of cutin and beneath that the so-called "cutinized lamella," where cutin-like substances are deposited in a cellulose layer, the upper layer of cutin is entirely devoid of a cellulose basis.

Considerable emphasis is laid upon these conclusions, which seem to the writer to be well founded. They indicate a point of view which must be taken into account by plant pathologists when considering the entry of parasitic fungi through the uninjured plant surface or when discussing the possible effect of spray fluids on the plant they protect.

Most parasitic fungi contain cytases, or cellulose dissolving enzymes, which are excreted at the growing tips and could probably disintegrate a membrane with a cellulose basis, as for instance many wood-destroying fungi disintegrate lignified membranes without digesting the lignin. But unless a fungus hypha penetrates through stoma or lenticel, it finds itself met at the surface of the plant by an unbroken lamella containing no cellulose fabric at all and which is chemically so composed that it would seem unlikely that ordinary hydrolysing enzyme action would decompose it.

It is therefore interesting to note that Blackman and Welsford⁽¹⁾ in the case of the parasitic fungus *Botrytis*, describe it as appearing to penetrate the cuticle by forcing its pointed tip forward under hydrostatic pressure, so that it wins its way through by mechanical displacement of the actual waxy substance of the cuticle.

The absence of cellulose in the external layers of the plant is a conception that does not seem to find favour with English writers, but possibly too much weight has been attached to the criticism of Cross and Bevan⁽³⁾ (*loc. cit.* p. 228). The views expressed in this English monograph, where cork is described as an "adipo-cellulose" or fatty substance allied to cellulose, are quoted in all English biochemical texts. Cross and Bevan briefly state van Wisselingh's point of view (although they quote him as "van Wissenburgh") and then brush it aside because, after removal of the suberin from cork by a special bisulphite process used on a macro-chemical scale, cellulose is indisputably present in the residue. But this is entirely beside the point. Reference to van Wisselingh's papers will show his frequent description of cellulose layers within cork cells, *internal* to the suberin lamella. The only point at issue is whether cellulose also occurs within the suberin lamella itself.

Van Wisselingh gives reasons, that seem very adequate, for concluding that it does not, and this being the case it will follow, unless the middle lamella has a cellulose basis, that the cork will provide an unbroken cellulose-free layer just as the cuticle does.

SUBERIN AND CUTIN NOT CHEMICAL ENTITIES BUT AGGREGATES OF SUBSTANCES

Van Wisselingh concludes that suberin is a mixture of substances because in his observations he sees these substances melting out of the lamella at different temperatures. In general he recognises two series of substances as present; the one series, melting at relatively

low temperatures and soluble to a large extent in boiling chloroform, he is inclined to regard as true fats; the other series, melting at higher temperatures or even decomposing before melting, or melting at lower temperatures after previous saponification, he would identify with the anhydride or condensation products of the suberogenic acids. But the mixture differs from plant to plant as might be expected. It has been possible to confirm one micro-chemical observation by macro-chemical methods. Van Wisselingh(17) describes the periderm of *Salix caprea* as remarkably aberrant under treatment by his method and notes that it never gives any indication of phellonic acid. Miss Rea has extracted the ground-up periderm of *Salix caprea* by Gilson's method and finds that no potassium phellonate at all can be identified in the alcoholic extract after saponification.

Cutin has not been so fully examined as suberin, but it is worthy of note that long prior to Gilson's work, Fremy and Urbain(7) as the result of the saponification of cutin (or "cutose" as they termed it) had obtained two organic acids that they described as "stearocutique" and "oleocutique." They had failed to identify any alcohol with which these acids were combined prior to saponification, and had noticed the tendency of these acids under various conditions to undergo profound modification in their properties, such as losing their solubility in fatty solvents, and in the case of the solid acid "stearocutique" a rise of melting point.

These reactions certainly suggest "cutinogenic acids" transformed in the formation of cutin in the same way as suberogenic acids are modified in forming suberin.

Furthermore, cutin, examined by van Wisselingh's method, can be shown to contain no trace of phellonic acid though other "cutinogenic" acids are present; this observation also is supported by some preliminary macro-chemical observations made by Miss B. Lee. In one case van Wisselingh(17 and 18) examined at different times the cuticle and periderm of the same plant, *Ilex aquifolium*, and a comparison of the results will show that in the same plant the substances forming cutin and suberin respectively do not seem to be identical.

We thus reach the conclusion that suberin and cutin are names for aggregates of substances which present certain characters in common, suberin being present in the median lamellæ of the walls of periderm cells and cutin as a continuous layer on the outside of the cuticle and in dispersed patches throughout the cutinised lamellæ below when these are present.

These substances consist of aggregates of organic acids, the suberogenic (or cutinogenic) acids, which are present to a small extent as glycerides or true fats, to a greater extent as condensation products or anhydrides of the acids. The differences between the suberin and the cutin of different plants, or between these two substances within the same plant will be due, in part to differences in their constituent acids, and in part to differences in the external and internal conditions prevailing whilst these acids pass over into the form that they assume in the mature suberin or cutin lamella.

A consideration of the work described above indicates many avenues of research opening before the investigator. On the one hand, only extended observation in which macro-chemistry and micro-chemistry both take their share can put the detailed knowledge of suberin and cutin on a broad basis, and until that is done the view outlined above, however satisfactory as an explanation, cannot be regarded as having a sufficient experimental basis.

On the other hand questions are suggested as to the methods by which the original lamellæ of suberin and cutin were formed. The acids described here as suberogenic are too empirically known yet to permit an elucidation of their constitution, though presumably they will readily admit of chemical derivation from some of the carbohydrates found within the plant (though by no means necessarily from celluloses).

The origin of the suberogenic acids has to be traced, and then we have to ascertain under what conditions in the plant these substances can assume the impermeable form, insoluble in fatty solvents, in which they are present in the mature suberin or cutin lamella. These conditions will be different from the methods of heating in sealed tubes adopted by Gilson and are probably already indicated by some of the literature referring to the conditions under which cork walls and cuticle are formed. It is for example very fascinating, in the light of the views expressed above, to read such a summary as that provided by Küster(12) of the conditions under which wound cork is formed, and to see that some conditions favour the early and extensive deposit of suberin on the cell arising from the newly formed meristem, whilst other conditions appear to impede its formation.

SUMMARY

1. Suberin is the name given to a substance present in the median lamella of the wall of periderm cells, between the middle lamella outside and the cellulose layer within; to this substance the

special properties of periderm, impermeability to water and resistance to sulphuric acid, are supposed to be due.

2. Cutin is a substance present as a continuous external lamella on the outer wall of the epidermis in leaf and stem to which is assigned the same *rôle* in reference to the cuticle.

3. The properties of these suberin and cutin layers may be jointly defined as insolubility in and impermeability to water, considerable insolubility in fatty solvents, great resistance to concentrated sulphuric acid, ready oxidation by nitric or chromic acids and ready solubility in warm alkali; they are stained by fat stains such as Sudan III or scarlet red.

4. In the layers in which suberin or cutin are present in the plant wall no cellulose can be detected; the importance of this conclusion to students of plant pathology is emphasised.

5. On chemical grounds suberin may be regarded as an aggregate of variously modified forms (condensation products or anhydrides) of certain organic acids, the suberogenic acids. The chemical constitution of these acids requires further elucidation before the problem of the origin of the suberogenic acids can be satisfactorily attacked.

To a small extent the suberogenic acids may be present in suberin in combination with glycerine as substances of the nature of true fats.

6. These suberogenic acids, some of which have been obtained crystalline and in the pure state, are usually soluble in fatty solvents at any rate on warming, but the anhydrides or condensation products formed from them are completely insoluble in fatty solvents and only give rise to the original suberogenic acids on saponification with alkali.

7. One of these suberogenic acids, phellonic acid, gives colour reactions with iodine reagents which are responsible for the erroneous impression that cellulose is present in the suberin lamella.

8. There is not the same experimental evidence in the case of cutin, but there is every reason for thinking that this is a similar aggregate of modified forms of "cutinogenic" acids.

9. Differences between suberin and cutin of different plants, or between the suberin and cutin of the same plant may be traced (1) to different organic acids and different proportion of those acids entering into the composition of the aggregate, (2) to the different conditions under which these acids have been transformed into the modified form in which they are present in the mature aggregate.

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STUDIES IN PHÆNOLOGY, No. 2, 1920.

BY FRANCIS DARWIN.

THIS paper is a continuation of my "Phænological Study" published in Nos. 9 and 10 of *The New Phytologist*, 18, November and December, 1919.

All my earlier observations (1917, 1918 and 1919) were made at Brookthorpe near Gloucester. The present series is indeed based principally on observations in that locality, but it also includes observations made at Cambridge and in the heathy country near Gomshall in Surrey.

The record of temperatures, which is the principal condition affecting the date of flowering, is (as in my former paper) taken from the "Weekly Weather Reports of the Meteorological Office." For the Gloucestershire observations I have taken the temperatures at Clifton, the observational centre nearest to Brookthorpe. For the Surrey observations I have taken the Wisley temperatures. For those in Cambridgeshire the temperatures at Cambridge.

Table I
Temperature from "Weekly Weather Report of the
Meteorological Office," 1920.

Week no.	Week ending	Mean temp. F.	Deviation from normal	Week no.	Week ending	Mean temp. F.	Deviation from normal
1	Jan. 10	38.7	+1.1	18	May 8	49.9	-0.6
2	17	45.1	+7.9	19	15	53.6	+1.4
3	24	43.9	+6.3	20	22	52.8	-1.0
4	31	42.0	+3.7	21	29	61.9	+6.8
5	Feb. 7	41.5	+2.6	22	June 5	57.4	+0.2
6	14	44.6	+5.8	23	12	56.4	-1.8
7	21	43.5	+4.5	24	19	60.0	+2.0
8	28	39.1	-0.2	25	26	61.1	+1.5
9	Mar. 6	44.3	+4.6	26	July 3	61.2	-0.2
10	13	39.8	-0.7	27	10	56.9	-5.0
11	20	43.9	+2.3	28	17	59.2	-3.0
12	27	48.9	+6.8	29	24	59.5	-3.1
13	Apr. 3	48.1	+3.7	30	31	57.3	-4.9
14	10	(No record)		31	Aug. 7	57.1	-5.4
15	17	49.2	+2.7	32	14	59.4	-1.6
16	24	48.8	+1.0	33	21	56.8	-3.8
17	May 1	47.9	-1.6	34	28	54.7	-5.4

I have also given the 1918 temperatures as being of interest in connexion with the comparison of the flowering dates of that year with those of 1920.

1918				1918			
Week no.	Week ending	Mean temp. F.	Deviation from normal	Week no.	Week ending	Mean temp. F.	Deviation from normal
1	Jan. 5	34.1	-5.6	27	July 6	63.8	+1.9
2	12	36.5	-2.5	28*	13	58.1	-1.9
3	19	39.6	-0.1	29*	20	60.3	-0.4
4	26	50.1	+9.8	30	27	60.2	-2.0
5	Feb. 2	44.6	+3.9	31	Aug. 3	64.1	+1.6
6	9	48.5	+8.1	32	10	62.7	+0.5
7	16	46.7	+6.2	33	17	62.9	+1.1
8	23	40.9	+0.7	34	24	64.0	+2.9
9	Mar. 2	40.9	0.0	35	31	58.3	-1.6
10	9	39.0	-2.3	36	Sep. 7	58.9	-0.2
11	16	45.1	+2.5	37	14	55.5	-2.7
12	23	47.0	+4.3	38	21	58.1	+1.4
13	30	46.3	+1.9	39	28	53.2	-1.7
14	Apr. 6	46.1	+0.2	40	Oct. 5	48.9	-4.0
15	13	46.8	+0.3	41	12	52.0	+0.8
16	20	40.1	-7.7	42	19	46.5	-3.4
17	27	49.6	+0.1	43	26	47.7	-0.2
18	May 4	49.9	-0.6	44	Nov. 2	52.0	+4.5
19	11	54.2	+2.0	45	9	45.5	-1.0
20	18	57.3	+3.5	46	16	43.3	-0.8
21	25	62.4	+7.3	47	23	38.7	-4.4
22	June 1	60.9	+3.7	48	30	46.6	+3.6
23	8	61.0	+2.8	49	Dec. 7	51.7	+9.7
24	15	56.5	-1.5	50	14	49.8	+8.0
25	22	55.3	-4.3	51	21	44.5	+3.7
26	29	57.0	-4.4	52	28	42.0	+1.7

The most obvious feature in the conditions ruling in 1920 is the relatively high temperature of the winter and spring. Thus from Jan. 4 to March 27, *i.e.* 12 observations, there are only two cases of temperatures below normal, viz. Feb. 28, -0.2 F.¹ and March 13, -0.7; the temperatures above normal varying from +1.1 to +7.9.

In the list (Table II) which follows, the first column gives the locality of the observation: thus the plants marked C were found in Cambridgeshire; those marked S were found near Gomshall in Surrey. The remainder (not marked with a letter) were found near Brookthorpe in Gloucestershire. Then follow (in separate columns)

¹ By -0.2 F. is meant a fifth of a degree below the daily mean temperature. The weeks are described by their later date, thus Feb. 28 means the week Feb. 21-28.

the current numbers of the observations (from No. 1 to No. 272), the date of observation and the name of the plant. Finally, for the sake of comparison, the flowering dates for 1917, 1918 and 1919 are given so far as they are available. Alongside these dates are placed the letters E and L, indicating earlier or later occurrence than in 1920.

Table II

No.	1920	Name	1917	1918	1919
1	Jan. 3	Ranunculus repens	—	May 10 L	May 15
C 2	8	Primula vulgaris	—	—	—
C 3	10	Tussilago petasites	—	—	—
C 4	21	Galanthus nivalis	—	—	—
C 5	22	Capsella Bursa-pastoris	—	—	—
C 6	Feb. 7	Ulmus campestris ♂	—	—	—
C 7	Mar. 3	Ranunculus Ficaria	Apr. 1 L	Feb. 10 E	Mar. 13 L
C 8	4	Lamium album	May 10 L	Feb. 16 E	Apr. 15 L
C 9	9	Taraxacum Dens-leonis	Apr. 25 L	Jan. 22 E	Mar. 15 L
C 10	11	Tussilago Farfara	—	Mar. 13 L	Apr. 5 L
C 11	21	Caltha palustris	Apr. 22 L	—	Apr. 9 L
12	24	Mercurialis perennis	—	♂ Fe. 4 E	♂ Mr. 14 E
13	24	Cardamine pratensis	May 2 L	Mar. 17 E	Apr. 7 L
14	24	Chrysosplenium oppositifolium	—	17 E	4 L
15	25	Fragaria vesca (wild)	Apr. 21 L	16 E	6 L
16	25	Lamium Galeobdolon	May 11 L	May 12 L	May 10 L
17	25	Oxalis acetosella	—	Mar. 16 E	Apr. 22 L
18	25	Primula veris	—	24 E	9 L
19	25	Nepeta Glechoma	May 2 L	21 E	12 L
20	26	Viola canina	—	—	22 L
21	27	Anemone nemorosa	—	Mar. 26 E	10 L
22	27	Viola odorata	—	13 E	Jan. 1 E
23	28	Lychnis diurna	—	Apr. 28 L	May 9 L
24	29	Scilla nutans	May 11 L	15 L	Apr. 22 L
25	29	Chærophylum sylvestre	18 L	16 L	May 6 L
26	30	Stellaria holostea	11 L	14 L	Apr. 22 L
27	31	Alliaria officinalis	14 L	8 L	May 6 L
28	Apr. 4	Vicia sepium	12 L	30 L	15 L
29	5	Heracleum Sphondylium	—	May 11 L	20 L
30	8	Arum maculatum	May 10 L	—	Apr. 28 L
31	9	Lychnis diurna (plenty; see No. 23)	—	Apr. 28 L	May 9 L
32	10	Vinca minor	—	—	—
33	10	Ranunculus auricomus var. depauperata	May 10 L	Apr. 12 L	May 10 L
34	10	Lamium maculatum	—	—	2 L
35	10	Veronica Chamædrys	May 12 L	Apr. 29 L	10 L
36	11	Paris quadrifolia	—	—	14 L
37	13	Pedicularis sylvatica	May 6 L	May 24 L	7 L
38	14	Plantago lanceolata ♀	12 L	Apr. 14 =	—
39	15	Acer Pseudo-platanus	6 L	May 9 L	May 9 L
40	16	Plantago lanceolata ♂	12 L	—	9 L
41	16	Geranium Robertianum	12 L	May 10 L	—
42	16	Chelidonium majus	—	—	—
43	17	Ranunculus acris	May 16 L	May 13 L	May 16 L

Table II—continued.

No.	1920	Name	1917	1918	1919
44	Apr. 19	<i>Veronica montana</i>	—	May 13 L	—
45	23	<i>Cratægus oxycantha</i>	—	1 L	May 18 L
46	23	<i>Ranunculus repens</i> (see Jan. 3)	—	10 L	15 L
47	24	<i>Asperula odorata</i>	May 15 L	17 L	24 L
48	24	<i>Veronica hederæfolia</i>	—	—	—
49	25	<i>Ajuga reptans</i>	May 7 L	Apr. 28 L	May 14 L
50	25	<i>Viburnum lantana</i>	—	—	—
51	25	<i>Heracleum sphondylium</i>	—	May 11 L	May 20 L
52	25	<i>Anthoxanthum odoratum</i>	—	9 L	15 L
53	25	<i>Pyrus Aucuparia</i>	—	—	19 L
54	28	<i>Trifolium pratense</i>	May 12 L	May 14 L	16 L
55	28	<i>Orchis morio</i>	23 L	—	15 L
56	28	<i>Ranunculus bulbosus</i>	—	Apr. 28 =	11 L
57	29	<i>Allium ursinum</i>	May 16 L	May 10 L	14 L
58	29	<i>Alopecurus pratensis</i>	—	9 L	17 L
59	29	<i>Ranunculus aquaticus</i>	—	—	12 L
60	30	<i>Veronica serpyllifolia</i>	—	May 9 L	15 L
61	May 2	<i>Trifolium agrarium</i>	—	17 L	18 L
62	3	<i>Poterium sanguisorba</i> ♂	Ju. 8 L	17 L	22 L
63	5	<i>Sanicula europæa</i> ♀	—	16 L	28 L
64	5	<i>Scrophularia nodosa</i>	Ju. 7 L	29 L	—
65	6	<i>Acer campestre</i>	—	5 E	May 24 L
66	6	<i>Cardamine amara</i>	—	—	26 L
67	12	<i>Geum urbanum</i>	May 28 L	May 14 L	21 L
68	12	<i>Geranium molle</i>	—	—	—
69	13	<i>Rhinanthus Crista-galli</i>	May 31 L	May 20 L	Ju. 5 L
70	13	<i>Polygala vulgaris</i>	Ju. 12 L	21 L	May 25 L
71	13	<i>Chrysanthemum leucanthemum</i>	May 30 L	22 L	28 L
72	14	<i>Ilex Aquifolium</i>	—	—	—
73	15	<i>Bunium denudatum</i>	—	—	May 20 L
74	16	<i>Sonchus oleraceus</i>	Ju. 21 L	—	Ju. 24 L
75	16	<i>Trifolium procumbens</i>	—	—	—
76	17	<i>Lychnis Flos-cuculi</i>	May 31 L	May 18 L	May 25 L
77	17	<i>Veronica Beccabunga</i>	29 L	17 =	26 L
78	19	<i>Rubus cæsius</i>	28 L	21 L	28 L
79	19	<i>Sonchus arvensis</i>	—	—	—
80	20	<i>Orchis maculata</i>	Ju. 11 L	May 24 ¹ L	Ju. 2 L
81	20	<i>Potentilla Tormentilla</i>	25 L	Ju. 10 L	May 27 L
82	20	<i>Sanicula europæa</i>	—	May 16 E	28 L
83	22	<i>Parietaria officinalis</i> ♀ stage	July 12 L	July 6 ² L	July 8 ² L
84	22	<i>Anthriscus Cerefolium</i>	—	—	May 23 L
85	23	<i>Bryonia dioica</i>	Ju. 9 L	May 30 L	♂ 28 L
86	23	<i>Euphrasia officinalis</i>	Aug. 3 L	—	July 28 L
87	23	<i>Hypochaeris radicata</i>	—	May 27 L	May 29 L
88	24	<i>Rosa canina</i>	—	21 E	Ju. 6 L
89	25	<i>Chærophylum temulum</i>	—	29 L	1 L
90	25	<i>Evonymus europæus</i>	Ju. 6 L	17 E	May 28 L
91	26	<i>Cornus sanguinea</i>	6 L	Ju. 2 L	Ju. 10 L
92	26	<i>Sambucus nigra</i>	10 L	May 21 E	May 29 L
93	26	<i>Orchis latifolia</i>	25 L	24 E	—
94	27	<i>Ægopodium Podagraria</i>	—	Ju. 6 L	Ju. 7 L
95	27	<i>Parietaria officinalis</i> ♂ stage	July 12 L	July 6 L	8 ² L

¹ Leaves not spotted.² No sex given.

Table II—*continued.*

No.	1920	Name	1917	1918	1919
96	May 27	Tamus communis	Ju. 6 L	May 28 L	Ju. 2 L
97	27	Sherardia arvensis	—	10 E	—
98	27	Lotus corniculatus	—	22 E	May 18 E
99	27	Lychnis vespertina	—	29 L	Ju. 8 L
100	27	Ranunculus arvensis	Ju. 3 L	—	24 L
101	27	Solanum Dulcamara	6 L	May 27 =	6 L
102	27	Onobrychis sativa	July 3 L	Ju. 6 L	2 L
103	27	Trifolium repens	Ju. 13 L	8 L	8 L
104	27	Hieracium pilosella	—	10	2
105	28	Galium aparine	Ju. 4 L	May 23 E	May 28 =
106	28	Scrophularia aquatica	—	—	—
107	28	Poa pratensis	—	—	—
108	28	Linaria cymbalaria (average)	Ju. 12 L	May 16 E	May 18 E
109	28	Lolium perenne	—	Ju. 10 L	Ju. 13 L
110	29	Potentilla reptans	Ju. 18 L	13 L	10 L
111	29	Potentilla anserina	—	May 27 E	4 L
112	29	Enanthe crocata	Ju. 18 L	—	13 L
113	29	Rumex acetosa	♂My 29 =	May 21 E	♂My 25 E
114	29	Urtica dioica ♂ (average)	♂Ju. 5 L	25 E	♂Ju. 2 L
115	30	Linum catharticum	8 L	25 E	6 L
116	30	Stachys sylvatica	May 30 =	Ju. 2 L	6 L
117	31	Helianthemum vulgare	Ju. 12 L	4 L	2 L
118	31	Avena pratensis	—	May 29 E	2 L
119	31	Rumex aquaticus (?)	—	—	—
121	Ju. 1	Neottia Nidus-avis	—	—	—
122	1	Plantago media	Ju. 6 L	May 22 E	Ju. 2 L
123	1	Tragopogon pratense	—	—	8 L
124	1	Milium effusum	—	—	May 27 E
125	2	Bromus arvensis	—	—	26 E
126	3	Dactylis glomerata	Ju. 10 L	Ju. 3 =	Ju. 1 E
127	3	Genista tinctoria	14 L	11 L	9 L
128	3	Briza media	11 L	6 L	6 L
129	5	Listera ovata	12 L	—	—
130	5	Polygonum aviculare	—	—	July 24 L
131	5	Rosa arvensis	May 21 E	—	7 L
132	5	Epilobium montanum	Ju. 12 L	May 28 E	Ju. 3 E
133	6	Ophrys apifera	July 11 L	July 16 L	—
134	6	Rubus fruticosus, var. glandulosus	—	—	Ju. 7 L
135	8	Spiræa ulmaria	Ju. 17 L	Ju. 5 E	Ju. 22 L
136	8	Lathyrus pratensis	8 =	17 L	12 L
137	8	Lapsana communis	18 L	16 L	20 L
138	8	Arrhenatherum avena- ceum	—	—	8 =
139	9	Galium cruciatum (ga- thered 11th in full flower)	—	—	—
140	11	Ophrys muscifera	—	—	—
141	11	Carduus palustris	Ju. 14 L	May 31 E	Ju. 9 E
142	12	Lonicera Periclymenum	—	—	21 L
143	12	Rubus fruticosus	Ju. 22 L	Ju. 24 L	July 10 E
144	13	Cephalanthera	—	—	—
145	13	Orchis pyramidalis	Ju. 21 L	Ju. 13 =	Ju. 22 L
146	13	Carduus acaulis	July 7 L	July 8 L	July 4 L

Table II—continued.

No.	1920	Name	1917	1918	1919
147	Ju. 13	Reseda luteola	July 27 L	—	Ju. 26 L
148	13	Thymus Serpyllum	Ju. 12 E	Ju. 24 L	Ju. 30 L
149	13	Sedum acre	—	—	—
150	14	Ligustrum vulgare	—	—	—
151	14	Lonicera Xylosteum	—	—	—
152	16	Centaurea nigra	Ju. 17 L	Ju. 6 E	Ju. 19 L
153	16	Cynosurus cristatus	—	13 E	—
154	16	Pimpinella Saxifraga, var. β dissectifolia	—	—	—
155	17	Papaver Rheas	Ju. 21 L	July 2 L	Ju. 21 L
156	18	Lactuca muralis	—	—	Ju. 23 L
157	19	Herminium Monorchis	—	Ju. 12 E	—
158	20	Geranium molle	—	—	—
159	20	Scabiosa arvensis	July 1 L	—	Ju. 25 L
160	20	Malva moschata	Ju. 21 L	Ju. 29 L	21 L
161	20	Geranium pratense	—	—	25 L
162	20	Circea lutetiana	July 26 L	Ju. 17 E	22 L
163	20	Carduus nutans	—	—	—
164	21	Hypericum hirsutum	Ju. 27 L	Ju. 20 E	Ju. 23 L
165	21	Silene pratensis	—	—	—
166	21	Campanula glomerata	Aug. 21 L	Ju. 19 E	—
167	22	Agrimonia Eupatoria	Ju. 27 L	—	Ju. 27 L
168	22	Vicia tetrasperma	—	—	—
169	22	Holcus lanatus	—	Ju. 16 E	—
170	22	Hordeum pratense	—	July 2 L	Ju. 22 =
171	22	Ranunculus sceleratus	—	Ju. 20 E	—
172	22	Anagallis arvensis	—	July 2 L	July 8 L
173	22	Matricaria Chamomilla	Ju. 12 E	10 L	Ju. 22 =
174	23	Scabiosa Columbaria	July 7 L	July 16 L	July 13 L
175	23	Verbascum nigrum	7 L	—	13 L
176	23	Centaurea scabiosa	Ju. 21 E	Ju. 29 L	Ju. 21 E
177	24	Medicago sativa (?)	—	—	July 12 L
178	25	Plantago major	July 13 L	July 5 L	Ju. 20 E
179	25	Campanula Trachelium	17 L	11 L	July 13 L
180	25	Asperula cynanchica	—	—	Ju. 30
181	25	Epilobium parviflorum	—	Ju. 30 L	July 10 L
182	27	Lysimachia Nummularia	July 5 L	July 7 L	2 L
183	27	Achillea Millefolium	7 L	5 L	Ju. 26 E
184	28	Chlora perfoliata	—	—	—
185	28	Valeriana officinalis	—	—	—
186	29	Carduus arvensis	Ju. 27 E	Ju. 30 L	Ju. 27 E
187	29	Æthusa cynapium	July 17 L	July 2 L	July 12 L
188	30	Aira cæspitosa	—	—	—
189	30	Glyceria fluitans	—	—	—
190	30	Galium verum	Ju. 21 E	Ju. 29 E	Ju. 21 E
191	30	Vicia cracca	—	July 7 L	—
192	July 1	Agropyrum caninum	—	10 L	—
193	3	Hypericum perforatum	—	9 L	—
194	3	Tilia	July 2 E	Ju. 30 E	July 9 L
195	4	Pyrola minor	—	—	—
196	4	Epilobium hirsutum	—	July 6 L	6 L
197	4	Convolvulus arvensis	Ju. 18 E	2 E	Ju. 22 E
198	4	Bartsia Odontites	July 17 L	12 L	July 11 L
199	5	Hypericum hirsutum	Ju. 27 E	Ju. 20 E	Ju. 23 E

Table II—continued.

No.	1920	Name	1917	1918	1919
200	July 5	<i>Apium nodiflorum</i> = <i>Helosciadium nodi-</i> <i>florum</i>	—	July 7 L	July 14 L
201	5	<i>Enanthe pimpinelloides</i>	—	—	—
202	6	<i>Convolvulus sepium</i>	Ju. 27	July 11	July 4
203	6	<i>Ballota nigra</i>	—	—	—
204	7	<i>Scabiosa succisa</i>	—	—	—
205	7	<i>Scabiosa arvensis</i>	July 1	—	Aug. 17
206	8	<i>Sedum album</i>	11	July 1	—
207	9	<i>Digraphis arundinacea</i>	—	6	Ju. 24
208	10	<i>Carduus acanthoides</i>	—	11	—
209	10	<i>Brachypodium pinnatum</i>	—	Ju. 24	Aug. 9
210	11	<i>Habeneria viridis</i> ¹	Aug. 11	—	July 28
211	11	<i>Senecio Jacobæa</i>	July 11	July 14	15
212	12	<i>Anthemis arvensis</i>	—	2	23
213	14	<i>Agrostis alba</i>	—	—	24
214	14	<i>Hypericum hirsutum</i> (not pale form)	Ju. 27	—	—
215	16	<i>Bromus asper</i>	—	July 16	July 22
216	16	<i>Ononis spinosa</i>	—	—	4
217	18	<i>Campanula rotundifolia</i>	July 6	July 9	—
218	19	<i>Helminthia echioides</i>	—	—	—
219	19	<i>Epilobium tetragonum</i>	—	—	—
220	21	<i>Origanum vulgare</i>	July 17	July 16	July 13
221	21	<i>Clematis Vitalba</i>	10	10	12
222	21	<i>Veronica Buxbaumii</i>	—	—	Apr. 22
223	24	<i>Arctium Lappa</i>	July 19	—	July 20
224	24	<i>Stachys Betonica</i>	11	July 13	18
225	28	<i>Inula Conyza</i>	Aug. 3	—	27
226	29	<i>Eupatorium cannabinum</i>	19	July 13	23
S 227	Aug. 4	<i>Rumex Acetosella</i>	—	—	—
S 228	4	<i>Calluna vulgaris</i>	—	—	—
S 229	5	<i>Artemisia vulgaris</i>	—	—	—
S 230	6	<i>Lycopsis arvensis</i>	—	—	—
S 231	6	<i>Linaria vulgaris</i>	—	—	—
S 232	6	<i>Ulex europæa</i>	—	—	—
S 233	6	<i>Erica cinerea</i>	—	—	—
S 234	7	<i>Hypericum humifusum</i>	—	—	—
S 235	7	<i>Teucrium scorodonium</i>	—	—	—
S 236	7	<i>Potentilla argentea</i>	—	—	—
S 237	7	<i>Spergularia rubra</i>	—	—	—
S 238	7	<i>Cuscuta europæa</i>	—	—	—
S 239	8	<i>Corydalis claviculata</i>	—	—	—
S 240	9	<i>Ornithopus perpusillus</i>	—	—	—
S 241	10	<i>Erodium cicutarium</i>	—	—	—
S 242	10	<i>Viola tricolor</i>	—	—	May 10
S 243	10	<i>Antirrhinum Orontium</i>	—	—	—
S 244	11	<i>Jasione montana</i>	—	—	—
S 245	11	<i>Trifolium arvense</i>	—	—	—
S 246	11	<i>Bartsia odontites</i>	July 17	July 12	July 11
S 247	12	<i>Solidago Virga-aurea</i>	—	—	—
S 248	12	<i>Hypericum perforatum</i>	—	July 9	—
S 249	14	<i>Galeopsis tetrahit</i>	—	11	—
S 250	14	<i>Ballota nigra</i>	—	—	—

¹ Found July 11 in advanced condition.

Table II—continued.

No.	1920	Name	1917	1918	1919
S 251	Aug. 16	Daucus Carota	July 11	July 12	July 18
S 252	17	Lamium amplexicaule	—	—	—
S 253	17	Fumaria officinalis	—	—	—
S 254	17	Sedum Telephium	—	—	—
S 255	17	Senecio sylvaticus ¹	—	—	—
S 256	19	Polygonum convolvulus	—	—	—
C 257	21	Calamintha menthifolia	—	—	—
C 258	23	Delphinium consolida	—	—	—
C 259	24	Humulus lupulus (? wild)	—	—	—
C 260	24	Lycopus europæus	—	—	—
C 261	24	Solanum nigrum	—	—	—
C 262	24	Sagittaria sagittifolia	—	—	—
C 263	24	Lycium barbarum	—	—	—
C 264	27	Stachys palustris	—	—	—
C 265	27	Inula dysenterica	—	—	July 23
C 266	28	Lychnis Githago	—	—	—
C 267	28	Lythrum salicaria	—	—	—
C 268	28	Mentha sativa	—	—	—
C 269	28	Selinum carvifolia	—	—	—
270	31	Scabiosa succisa	—	—	Aug. 17
271	Sep. 1	Sison amomum	—	—	July 28
C 272	2	Gentiana Amarella	Aug. 20	—	Aug. 28

¹ No. 259 should perhaps have been noted on a slightly earlier date.

The letters L and E in Table II show (as above stated) the broad relations between the dates of 1920 and those of 1917, 1918 and 1919. The letter L means that the species so marked flowered later than in 1920; in the same way E indicates earlier flowering.

What is at once obvious is that the 1917 dates are universally later than the corresponding entries for 1920. The same thing is, roughly, true of 1919, there being only three cases in the first 100 which are earlier than 1920, and one plant flowered on the same day in 1919 and 1920. In 1918 the case is different. Taking the same material, viz. No. 7 to No. 107, we have:

March 3–April 11	12 E	11 L	
April 13–May 13	1 E	21 L	2 equal
May 14–May 27	8 E	18 L	2 equal
The sums are...	21 E	50 L	4 equal

We see that the cases in which the spring flowers of 1918 (March 3–May 27) were earlier than those of 1920 are 21 in number: the cases in which 1918 is later being 50; there are also four cases

of equality. Thus out of a total of 75 cases 28 per cent. are early, 67 per cent. late and 5 per cent. are equal.

It should be noted that among the early cases a majority occurs between March 3 and April 11.

In conclusion, it may be pointed out that there is a general resemblance between 1920 and 1918, inasmuch as the springtime flowerings are early in both these years. And this fact we must connect with the relatively high temperatures ruling in the spring of these two years. Thus from the week ending Jan. 26, 1918, to week ending Feb. 16 we have deviations from the normal of + 6.2 to + 9.8. And, again, from March 16 to April 13 the deviations are + though not so well marked as those quoted.

The early spring flowerings of 1920 also correspond with the relatively high temperatures ruling from the week ending Jan. 10, 1920, to that ending Feb. 21, 1920.

THE THEORY OF GEOTROPIC RESPONSE

By V. H. BLACKMAN

IN recent numbers of this journal (19, pp. 49-63 and 208-212) Professor Small has put forward a theory to explain the geotropic response in plants, and particularly the difference in the behaviour of the stem and the root. This theory rejects the movement of comparatively large cell particles (such as starch grains) as the first step in the chain of processes which lead to geotropic curvature, and relates the response to changes in the plasma itself under the action of gravity. The particles or drops forming the disperse phase of the colloidal protoplasm are supposed to be lighter than the surrounding fluid (continuous phase) and to rise to a marked extent under the action of gravity, a phenomenon which is described as "creaming." As these particles are electrically charged their movement should produce a difference of potential or alter the difference of potential already existing in the cell. The particles are supposed to be electro-positive in the root and electro-negative in the stem; the differences of potential produced, and the electric currents to which they give rise, would thus be in opposite directions in the two organs. These currents are supposed to affect the permeability and growth of the cells and so bring about the geotropic curvature.

The nature of the geotropic response (even if the statolith theory be accepted) is one of the most obscure of physiological phenomena,

especially in the diversity of reaction of the stem and the root. Professor Small has therefore shown great courage in attacking the problem and great ingenuity in working out his theory. Our knowledge of the colloidal and electrical conditions of the living cell is unfortunately so slight that any theory of this kind must be highly speculative, but in considering the theory as a working hypothesis a number of serious difficulties appear to arise, some of which are here put forward.

(1) One difficulty—and it is naturally a fundamental one—is as to the actual occurrence of the “creaming” effect. As is well known, there is no obvious settling of colloidal solutions; gold solutions made by Faraday more than sixty years ago are still to be seen at the Royal Institution. Yet, on the other hand, far smaller particles, for example, gaseous molecules, do settle to some extent as is clearly shown by the decrease in air pressure as we rise above sea-level. The explanation of this apparent discrepancy is that all particles settle to some degree, whether they are gaseous, molecules, ultra-microscopic particles, or microscopic particles; but the degree to which they settle (*i.e.* the alteration of concentration with height) depends on the volume of the particles and their relative density. The atmosphere follows what is called the exponential “rarefaction law”; if we go up six kilometres the density (*i.e.* concentration of molecules) falls to one-half, if we go up another six kilometres it falls to one-quarter, and so on. Einstein in 1905, and independently Perrin in 1908, showed that if the Brownian movement of particles is due to molecular bombardment the distribution with height of such particles must follow the same law. The amount of settling or rising for any given height—whether the particles are gaseous molecules or colloidal particles—will depend on the volume of the molecules or particles and their density. Perrin¹ was able to demonstrate by examination of carefully prepared colloidal solutions of gamboge and mastic that the particles did obey the “rarefaction law,” for at each equal step upwards the concentration of particles decreased in geometrical progression. Perrin, in one of his experiments, found that for gamboge particles of radius 0.21μ the concentration was halved for each rise in height of 30μ .

From data such as these of Perrin's we can obtain some idea of the degree of settling or “creaming” which would result from the action of gravity on the colloidal particles of the protoplasm. Perrin worked with microscopic particles; the protoplasmic

¹ J. Perrin, *Brownian Movement and Molecular Reality*. (English translation), London, 1910; also *Atoms* (English translation), London, 1916.

ones in question are ultra-microscopic, and we may assume their diameter to be $1/10$ th that of Perrin's gamboge particles—an assumption which does not seem to err on the side of smallness. The relative density may be assumed to be the same as the gamboge particles (0.207), though that of the protein drops of the cell would probably be less. As the volume of the particles is only $1/1000$ th that of the gamboge ones, it would require a cell 30 millimetres high to obtain a difference of concentration of 50 per cent. between the top and bottom. Taking a meristematic cell of the root as 30μ across, it is easy to calculate that for ultra-microscopic particles of the size suggested the drop in concentration between the top and bottom of the cell would be only 7 parts in $10,000$, or 0.07 per cent.; if the cell is taken as 50μ broad it is still only 11 parts in $10,000$, or 0.11 per cent.¹ There is thus no reason to believe that the differences of concentration of particles in the cell produced under the action of gravity would be other than negligible in amount, and in fact so small that the term "creaming" can hardly be applied. It seems inconceivable that such small differences in concentration could produce the marked electrical effects required by Professor Small's theory.

(2) There is another great difficulty which the theory presents, namely, that of the factor of time. It is easy to show that even the very small movement—which, as pointed out above, is all that can be expected of the cell particles—would take place so slowly that it could not be a link in the chain of processes which controls the rapid geotropic reaction of the stem and root. Perrin, working with the large colloidal particles of radius 0.21μ , allowed three hours for the completion of the process. With decrease in size of the particles or increase in viscosity of the medium, however, the time taken to reach equilibrium increases. In another of Perrin's experiments with rather larger particles (0.38μ radius) and a highly viscous medium (viscosity 125 times that of water) the time taken to reach equilibrium was "several days." One can calculate from this that particles no smaller than of 0.034μ radius moving in a watery medium would also take days to reach equilibrium. As by the author's hypothesis the particles are ultra-microscopic and so must be of this order of size and probably smaller, and the cell medium in which they move is more viscous than water and possibly highly viscous, *the time taken to settle down or rise up under the action of gravity must also be of the order of days.*

¹ Even if the diameter of the ultra-microscopic particles of the protoplasm be taken as only $1/5$ th of that of the gamboge particles, the difference in concentration in cells 30μ and 50μ broad would be only 0.6 per cent. and 0.9 per cent. respectively.

Professor Small refers to Perrin's work on Brownian movement (1910, *loc. cit.*) in which it was demonstrated that the rate of fall or rise of colloidal particles under the action of gravity can be calculated from Stokes' law. The conclusion, however, he draws from Perrin's work that in the cell the "creaming is governed by Stokes' law" (footnote, p. 52) is certainly mistaken. Once it is recognised that colloidal particles obey the "rarefaction law" it is obvious that Stokes' law, which applies to freely falling particles, is only followed when the particles are far removed from their equilibrium distribution. Perrin makes this point clear (*loc. cit.* 1910, p. 34), and in investigating the application of this law to the comparatively large particles of gamboge he used a tube several cms. long. The small ultra-microscopic granules within the narrow confines of a cell only 0.05 mm. in height must always be little removed from their limiting distribution, as demonstrated above; they therefore cannot fall freely and Stokes' law cannot apply. In order that the plasma particles should be under the same conditions as Perrin's gamboge particles a cell at least a *metre* high would be required¹.

The considerations put forward above indicate that any rearrangement of the cell particles which might occur under the action of gravity would be exceedingly small in amount, and would be accomplished very slowly. The geotropic response, on the other hand, is a particularly rapid process. Under ordinary conditions a "presentation time" as low as two minutes has been observed, and the "excitation time" is certainly very much less. Professor Small has himself described earlier a geotropic reaction appearing as a change in electrical conductivity of the cells of the root tip which is to be observed in as short a period as 20 seconds after the organ is placed horizontal. Bose (*Trans. Bose Inst.* 11, pp. 500 and 452, 1919) found in one case a large electric response occurring one second after the horizontal position was reached, and in another case the maximum deflection was attained in 90 seconds. It would seem impossible to correlate active electrical and mechanical reaction occurring in a few seconds or minutes with a redistribution of plasma particles so limited in extent and requiring days for its accomplishment.

(3) There is also another difficulty which would seem to arise if

¹ Even if the cell particles were able to move freely, their small size would render their rate of rise, as calculated by Stokes' law, very low. If we assume that their radius is 0.021μ and that their relative density is the same as Perrin's gamboge particles (*i.e.* 0.207), and that the viscosity of the protoplasmic medium in which they move is only twice that of water (0.01), the rate of ascent would be only 1μ in 10,000 secs. = 2.8 hours. The rate would really be slower as the particles by their movement are doing work in producing a difference of potential in the cell.

the "creaming" occurred and a difference of potential were produced. Since the potential, which is assumed to result from the movement of the particles, is, *ex hypothesi*, being dissipated by the current which it produces in the organ, it should soon disappear when the "creaming" is complete. In an organ kept horizontal it would seem that the difference of potential produced by the "creaming" should soon cease to exist, and, as the current produced by this difference is by the theory the cause of the curvature, the organ should soon cease to react to gravity. A root compelled to grow for a time through a short horizontal tube should, on becoming free at the other end, have lost the power of geotropic response. The same difficulty arises as to the origin of the currents in the axial and lateral organs, which, Professor Small holds (*loc. cit.* pp. 59 and 209), explain the relation of secondary branches to the primary root and stem. The normal polarisation of the plasma membrane will not produce currents in uninjured cells. Also the apical meristems of, for example, the normal undisturbed root system, would have been "creamed" from their first origin, so no difference of potential should arise in their case.

The recent experiments of Bose (*loc. cit.*) do seem definitely to support the statolith hypothesis in the case of the stems he examined. When the stem is horizontal, a high E.M.F. is rapidly attained; it is at its maximum when a lead is taken from the endodermis, and no current is obtained when the electrode is in the centre of the stem; also he found that when the starch grains had disappeared from the endodermis there was no electric response of the stem. Bose also described a striking case where there was no electric response when the flower stalk of *Nymphaea* was displaced by 33° , but "when this critical angle was exceeded by a single degree there was a sudden precipitation of geo-electric response" (p. 500). This would seem to be consistent with the sticking and displacement of large particles, but is quite inconsistent with the movement of ultra-microscopic ones in the protoplasm.

That the hydrogen-ion concentration of the protoplasm plays an important rôle in cell processes, and that differences in this respect may explain the difference of geotropic response of the root and stem as assumed by the theory, would seem very plausible. If, however, a reaction which takes place as rapidly as the geotropic response is to be correlated with the movement of particles in a viscous medium it would seem certain that such particles must be comparatively large and heavy, not ultra-microscopic.

REVIEW

Practical Plant Biochemistry By MURIEL WHELDALD ONSLOW.
(Cambridge University Press, 1920.) pp. i + 178. Price 15s.

The author, who is well known from her previous book, *The Anthocyan Pigments of Plants*, points out in the preface that the volume is compiled primarily for students of Botany who have some knowledge of Organic Chemistry. Its title might suggest that it is solely a laboratory manual, but, in fact, it combines in a very useful way a small text-book of plant chemistry with directions for numerous practical exercises. There are ten chapters, of which six deal with the chief classes of chemical compounds to be found in plants, namely, carbohydrates, fats and lipases, aromatic compounds and oxidising enzymes, proteins and proteases, glucosides and glucoside-splitting enzymes, and the plant bases, the last chapter being a very useful addition. There are, besides, an introductory chapter, and chapters on the colloidal state and on enzyme-action, and also a chapter on carbon-assimilation which includes a useful *résumé* of the chemistry of chlorophyll. Here and there we find a brief discussion of a few physiological problems such as those of the first formed sugar of the green leaf, and of the synthesis of fats, and a short but useful list of references is appended to each chapter. It is satisfactory to find in the chapter on carbohydrates a short account of the pentosans, the importance of which in succulent plants is being more and more recognised. In this chapter, however, mention might have been made of all the sugars which are referred to later in connexion with the splitting of glucosides. The statement, also, that "all these structural elements [of the plant] can be translated into terms of chemical compounds" seems to savour more of expectation than of present-day realisation. The colloidal state and enzyme-action are somewhat cavalierly treated and the references to surface action are almost too brief to be helpful; these two chapters might usefully be expanded in a later edition. These minor criticisms apart, the book is certainly a marked success, and the author is to be congratulated on an introduction to plant biochemistry which is interesting, clear and compact, and which for the first time makes the subject easily accessible to the average botanist. The practical instructions given should also help to improve in universities the quality of the laboratory work in plant biochemistry.

V. H. B.

THE ANGLO-AMERICAN UNIVERSITY LIBRARY
FOR CENTRAL EUROPE

IT is now well known that the scientific men of Central Europe are severely handicapped in their efforts to obtain scientific literature from foreign countries owing to their poverty and to the very unfavourable rates of currency exchange. An organisation has been formed to establish libraries for Central Europe from which books and papers are to be distributed to the university professors and teachers who require them. Eight centres of distribution have

at present been established: Berlin, Budapest, Frankfurt, Göttingen, Munich, Krakow, Prague and Vienna, and several consignments of literature have already been sent to these centres. The organisation for the United Kingdom is under the control of a strong committee under the presidency of Viscount Bryce and the chairmanship of Sir William Beveridge.

Gifts of separate copies of scientific papers which have appeared since 1914, as well as spare copies of books and periodicals, would be very useful, and should be sent to the Hon. Secretary, Mr B. M. Headicar, London School of Economics, Clare Market, London, W.C. 2, by whom they will be gratefully acknowledged. Cheques and money orders to help the scheme should be sent to Lieut.-Col. George Schuster, at the same address.

We appeal to our readers and contributors to do what they can in aid of this admirable scheme of help to our fellow-workers in Central Europe.

THE BRITISH COMMITTEE FOR AIDING MEN OF LETTERS AND SCIENCE IN RUSSIA

A STRONG committee of British men of science and letters, including the President of the Royal Society, the Editor of *Nature*, Mr Ernest Barker, *et al.*, has been formed to help Russian men of science and letters, who are suffering great privations and have had practically no access to European literature since the Revolution. Some members of the British committee have recently been in direct communication with the Russians, and a comprehensive list of books and other publications, which they most urgently need, has been made. The purchase of these and the cost of transmission will involve a considerable expenditure, and the British committee are asking for subscriptions, which should be sent to the Treasurer, Dr C. Hagberg Wright, British Science Guild Offices, 6, John Street, Adelphi, London, W.C. 2.

It is understood that there will be no obstacle to the transmission of the literature to the House of Science and the House of Literature and Art in Petrograd, the present headquarters of the Russian savants. This scheme also we cordially commend to those of our readers who can afford financial help.

ERRATA from XX. 1.

Page 5, line 16. For *Rochea* read *Kleinia*.

Page 23, third paragraph, third line. For "phellonic" read "phloionic," and
for "considerable" read "small."

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PERMEABILITY

By WALTER STILES

CHAPTER I

INTRODUCTION

IN the study of the living organism there presents itself a well marked group of problems arising from phenomena which can be included under the term "Permeability." Every organism receives from its environment in some form or another substances which enter into its body and which either as such, or after undergoing physical and chemical change and working up into new combinations, may be carried to every part of the organism. The problems concerned in this intake into the organism of substances from the surroundings, and their passage out from the cell into the external medium, and the translocation of substances from cell to cell in the body of the organism, may be spoken of broadly as problems of permeability. It must be admitted at the outset that the word permeability in biology is largely a cloak for ignorance. When the physical chemist speaks of the permeability of a membrane, he refers to the capacity of the membrane for allowing substances to pass through it, and this is the logical and correct significance of the term. But this is not always what is meant when the physiologist speaks of the permeability of protoplasm or the permeability of an organic cell. Sometimes he means the capacity of a substance to pass into or out from the cell, sometimes its capacity to pass through one particular part of the cell, as, for instance, the protoplasm, or the surface layer of it. The cell is a complex structure, and in the present state of our analysis it is rarely possible to localise the seat of any cellular phenomena exactly, although it must be one

of the objects of research to do so. While therefore we must aim at a stricter use of the term permeability so as to conform to its usage in physical chemistry, at present our knowledge simply does not allow us to do so when we are dealing with the problems of the cell.

Research on the problems involved has proceeded along two rather distinct lines. In one, the whole living organism has been the unit of experimentation, while in the other isolated cells and tissues have been employed. In the case of unicellular and other small organisms the difference between the two groups of methods disappears. The difference between the two lines of attack is, however, very obvious in the case of work on higher plants where the methods of the first mode of attack are for the most part those of pot culture and water culture. In the hands of a number of plant physiologists from Woodward in 1699 onwards, research by these methods led to fundamentally important discoveries in regard to plant nutrition, and in the hands of Sachs and Knop about 1860 it was used to demonstrate successfully the elements essential for the nutrition and development of plants, the absorption of water and dissolved substances from the soil and the absorption of gases from, and their excretion into, the air. The methods as employed to-day have provided a quantity of empirical information on the relation between the amount of growth of plants and the constitution of the medium external to their roots; as far as permeability problems are concerned they have not led us much further than the experiments of Sachs, Knop and other workers of their time, who showed by water-culture experiments that plants were capable of absorbing certain substances through their roots, while ash analyses showed that the constituents of these substances were capable of passing through the tissues to remote parts of the organism. These methods, in short, afford no data in regard to the intake of salts by the plant and the subsequent movement of the absorbed substances, beyond the information that these take place in certain cases. Nevertheless in the past they yielded results of the first importance for our subject, and there is no reason to suppose that, with suitable modification, their period of usefulness is over.

In the animal organism similar considerations hold. From considerations based on the whole organism as a unit it early became clear that some substances could penetrate through certain cells and become absorbed into the animal, while others could not. But, generally speaking, this line of attack has not afforded quantitative

data bearing on permeability, and so has not greatly helped towards a clear insight into the problems involved.

It is to the second line of attack that we owe most of our knowledge of permeability. The use of isolated cells or isolated pieces of tissue allows the employment of more exact methods and more careful control than can always be obtained in experiments with the whole organism. Such material as roots or stems and slices of storage tissues such as tubers or fleshy roots in the case of plants, blood corpuscles, eyes, pieces of muscle in the case of animals, have formed successful objects of experimentation. The elimination of error arising from variability among different individuals, the so-called "biological error," is possible with this mode of attack, and by a proper method of experimentation results can be obtained approximating in exactness more nearly to those of physics and chemistry than is possible when whole plants or animals form the experimental object, and such results are reproducible.

As tissues differ in the form of the cells which compose them so they differ also in their functions, and it is reasonable to suppose there is no more uniformity in regard to permeability than in regard to other functions. By the use of isolated tissues we are thus on the way towards a physiological analysis which is not possible when the whole organism forms the experimental unit.

The problems of permeability are problems of general physiology; they are common to all life, plants and animals, the lowest and the highest. They are problems of the cell and of the organism as a whole. While in this account of our present knowledge of permeability we shall deal mainly with plants, we shall thus have occasionally to refer to work on animals, as this may often be helpful in understanding the conditions in plants, while the results of investigations dealing with plants from very different groups of the plant kingdom and with both whole plants and parts of plants will have to be considered.

CHAPTER II

THE SYSTEM INVOLVED

THE problem with which we are presented is then to discover the laws governing the penetration of substances into and through the living cell. It is obvious that it is of first importance to understand the system involved. Both from a chemical and physico-chemical point of view this is a very difficult matter. The essential of all living cells is protoplasm. The structure of this and its elementary properties have been described as among the most difficult problems with which the biologist has to deal (Bayliss, 1915). In its simplest form in *Amoeba* or *Myxomycetes*, the general body of the protoplasm, that is, the cytoplasm apart from enclosed granules, appears as a clear viscous fluid, apparently structureless, capable of changing its form under the influence of external conditions, but remaining quite distinct from, and without any tendency to mix with, the medium external to it.

It has been urged that protoplasm behaves as a liquid (Bayliss, 1915). This is shown by (1) the fact that drops of water enclosed in it assume a spherical form, (2) Brownian movement¹ observed by R. Brown in 1827 (R. Brown, 1866), (3) the action of an electric shock under the influence of which, an amoeba, for instance, tends to form a sphere (Kühne, 1864), (4) the behaviour of *Myxomycetes* (Lister, 1888).

Under ordinary powers of the microscope certain parts of the protoplasm are visible as denser specialised organs of the cell: the nucleus, numerous small granules, and in plants the plastids. Apart from these, under the ultra-microscope (dark-ground illumination) the apparently homogeneous cytoplasm is observed not to be homogeneous, but to contain a great number of minute particles (Gaidukov, 1906-1910; Mott, 1912; Price, 1914). This is the condition characteristic of colloidal solutions, and from ultra-microscopic observation it would seem reasonable to conclude that the cytoplasm is frequently a colloidal liquid system or hydrosol.

Cytoplasm then is not homogeneous, but consists of a denser phase dispersed through the watery dispersion medium. It thus

¹ Brownian movement of particles visible under the ordinary microscope cannot be observed in all cells or organisms (Seifriz, 1920).

has a structure, but it must be emphasized that this structure is ultra-microscopic and not coarse enough to be observed under the ordinary microscope as was at one time thought. The production of a reticulate structure in the cytoplasm when treated with fixing and staining agents led to the view that cytoplasm possessed a net structure visible under the microscope. Even if the views of some earlier writers more or less dimly suggest the opinion that cytoplasm is in the colloidal condition, and while Bütschli (1892) suggested that living protoplasm has the structure of a microscopic emulsion, it was Hardy (1899) and A. Fischer (1899) who showed that the structure of cytoplasm after treatment with fixing agents could be made to vary according to the treatment during fixation. The conclusion to be drawn is obviously that the reticulum generally observed is the result of the fixing, and for the reasons already given it must be held as undoubtedly true that cytoplasm is essentially a colloidal system.

Although in many cases the body of the cytoplasm is a sol, there is a certain amount of evidence that in many cells the cytoplasm may be in the more solid gel condition. Thus Bayliss (1919) says: "That there are possibilities of the formation of membranes, doubtless of a gel nature, within the protoplasm of a cell is shown by the fact that different reactions can take place at the same time in different parts of the cell, notwithstanding the general liquid nature of its contents." Gaidukov (1910) and Price (1914) by ultra-microscopic observation find that in some cases Brownian movement in the cell may cease, and suppose the protoplasm in these cases has taken on the state of a gel.

Price concludes that protoplasm can, and often does, exist in the gel state, and in this state may be active. Bayliss has been able to bring about the cessation of Brownian movement by weak electrical stimulation and relates the phenomenon with functional activity (1919). Chambers (1917), by microscopic observations made on dissections of living cells, also comes to the conclusion that in the ova of a number of marine organisms (*Asterias*, *Arbacia*, *Echino-arachnius*, *Cerebratulus*, *Fucus*) and in the germ cells of certain insects (*Periplaneta*, *Disosteira*, *Anasa*) as well as in protozoa, the cytoplasm usually exists as a sol. On the other hand he considers that in adult somatic cells, including nerve cells and muscle fibres, the protoplasm forms a more or less rigid gel. Leucocytes however possess a cytoplasm closely resembling that of germ cells. Seifriz (1918, 1920) also, in a similar series of observations on *Myxomycetes*,

oogonia, egg cells and embryos of *Fucus*, *Spirogyra*, *Vaucheria*, *Rhizopus* and *Zygorhynchus*, pollen tubes of *Iris versicolor*, *Lathyrus maritimus*, *Erythronium revolutum*, protozoa, and ova of *Echinoarachnius*, finds the viscosity of protoplasm varies greatly in the different cells examined. Young *Fucus* oogonia and embryos and the streaming protoplasm of Myxomycetes were the most liquid, the mature and resting eggs of marine organisms were the most viscous. This author however is of opinion (1920) that great caution should be exercised in using viscosity as the only criterion of a sol or gel condition of the protoplasm.

Price decides that in resting spores, for example, those of *Mucor*, the protoplasm is in the gel condition, but that on germination it becomes a sol. Changes in the consistency of *Fucus* eggs during maturation and fertilization are described by Seifriz (1918), while the same writer (1920) states that as a myxomycete prepares to fruit the protoplasm increases in viscosity until it becomes in consistency a gel. Seifriz has observed such changes in protoplasmic viscosity which are reversible. "The viscosity of protoplasm is not fixed, for it varies in different organisms, in the same organism at different times, and even in different regions of the same organism at the same time" (Seifriz, 1920).

Thus even where the bulk of the cytoplasm is a sol there is a considerable quantity of evidence that the surface layers of the cell plasm may be in the more solid gel condition. Loeb (1906) says confidently: "It is a general rule that every free cell is surrounded by a solid film." He instances as evidence of this the length of the pseudopodia of rhizopods, which is so great that if the pseudopodia were entirely liquid they would fall apart into droplets. As protoplasmic streaming takes place in the interior, the solid part of the pseudopodia must be at the surface. Ramsden (1894, 1903) and Traube (1867) have shown the formation of solid membranes at the surface of hydrosols.

In cells in which Chambers decides the cytoplasm is in the sol condition he finds the peripheral layer very dense in consistency as compared with the interior of the cell, the outer layer merging insensibly into the general body of the cytoplasm. This surface layer is particularly well marked in protozoa, for example, in *Paramoecium*. Chambers concludes that the surface layer is a "highly extensile contractile and viscous gel," which if damaged may be automatically repaired. In adult somatic cells where the interior cytoplasm is judged to be in the gel state it is not possible

to demonstrate an outer layer differing in consistency from the rest of the cell. Price from ultra-microscopic observations states that the presence of an outer layer of the protoplasm differentiated from the interior seems quite definite. In any case it must be emphasized that the surface of the cell constitutes a boundary between two immiscible phases and as such the properties of the surface will differ greatly from those of the main bulk of the protoplasm. Indeed, the surface layers of the protoplasm are generally regarded as differing so much from the rest of the protoplasm as to have different permeability properties. The evidence for and against this view will be more suitably dealt with in a later chapter when the cell membranes are considered in more detail.

The chemical composition of protoplasm is rendered difficult of determination as the methods of chemical analysis in themselves necessarily change the living matter into something essentially non-living and therefore different. Chemical analysis has however provided some information regarding the components of the living substance. Water often comprises about 80 or 90 per cent. of the total weight of protoplasm. The classical analysis of Reinke and Rodewald (1880) of the plasmodium of the myxomycete *Fuligo varians* showed that about 60 per cent. of the dry weight of the protoplasm consisted of proteins, about 20 per cent. of carbohydrates and fatty substances, while the remainder consisted of amino-acids and other organic acids, various organic bases and inorganic salts. In different samples of protoplasm the relative quantities of the substances of which it is composed vary; proteins may form as little as 40 per cent. of the total dry matter. It has been a matter of controversy whether cytoplasm is a single definite substance peculiar to living matter, or whether it is composed of a large number of relatively simpler substances. In the elaboration of the former view the molecule or "biogen" is held to consist of a stable central nucleus to which are attached side-chains capable of undergoing various chemical transformations such as oxidation and reduction, and to which the activities of the cell are due (Verworn, 1903). The opinion that protoplasm comprises a large number of different substances is more generally held to-day. Thus Czapek (1911) remarks: "But we have to concede that the chemical nature of protoplasm is not founded upon the peculiarities of one particular substance which is characteristic of living protoplasm. There are, we are certain of it, a great number of constituents of protoplasm which form the substratum of cell-life."

Hopkins (1913) thinks the biogen molecule theory "as inhibitory to productive thought as it is lacking in basis." Bayliss (1915) regards the biogen theory as "an example of the efforts of a certain school of physiologists to explain by purely chemical laws, such as mass action, facts which admit of a simpler explanation, if physical phenomena are also taken into account."

We have to think of protoplasm not merely as an intimate mixture of a large number of substances, but as having a complex organisation so that the cell is rather an organ with an intricate minute structure, and at the same time different reactions can take place in different parts of the same cell. "Protoplasm is an extraordinarily complex heterogeneous system of numerous phases and components, continually changing their relations under the influence of electrolytes and other agents" (Bayliss, 1919). For a further discussion of this question reference may be made to the writings of Verworn, Czapek, Hopkins and Bayliss cited above.

Some parts of the protoplasm are clearly differentiated from the rest. The most important of these is the nucleus which has been observed in all plant and animal cells with very few exceptions. From its appearance in fresh cells and from its reaction to stains it obviously differs from the cytoplasm that encloses it. There is considerable evidence that it is much richer in nucleoproteins, which contain phosphorus, than the surrounding cytoplasm. The observations of Gaidukov and Price with dark-ground illumination point to the fact that the nucleus is in the gel condition. Kite (1916) and Chambers (1917) conclude on the contrary that the resting nucleus of the ovum is in the sol state. Price made out a definite limiting layer separating the nucleus from the cytoplasm, but he thinks it possible that this, the so-called nuclear membrane, may be no more than the surface of separation between the cytoplasmic hydrosol and the nuclear hydrogel. The intimate connection between cytoplasm and nucleus as regards cell activity is so well realised that it needs no further emphasis.

The plastids occur only in plants, and even then not in all plants. They are absent, for example, from the Fungi. Like the nucleus they are sharply differentiated from the rest of the cytoplasm and appear to contain much protein. In addition they often contain pigments (chlorophyll, xanthophyll and carotin) our knowledge of the composition of which is due to the persistent researches of Willstätter (1913). According to Price the chloroplasts of *Spirogyra*, *Elodea* and other plants are, like the nucleus, in the gel state.

The small granules occurring throughout the cytoplasm may be in some cases tiny non-living inclusions and in other cases small plastids or other living bodies. Chemically they may be composed of fat, glycogen, protein, etc., as shown by microchemical tests. Chambers divides them into microsomes and macrosomes, the former being very minute and considerably less than $1\ \mu$ in diameter, while the macrosomes vary from 2 to $4\ \mu$ in diameter and are circular, oval or polygonal in shape. The macrosomes are the alveolar spheres of Wilson (1899), with whom Chambers disagrees as regards the complete gradation from macrosomes to microsomes. Chambers regards the two sets of bodies as quite distinct; the macrosomes are the most easily injured, and the microsomes are the most resistant, of all cell structures. Into this controversy we need not enter here, nor yet into the question to what extent the microsomes are identical with mitochondria. Those interested should consult the very numerous papers on this subject recently published by Guilliermond (1916-1921).

Whereas in animal cells the protoplasm frequently occupies the whole of the cell space, in plants this is the case only with meristematic cells. As the cell passes out of the meristematic condition small inclusions of less viscous consistency become obvious in the general mass of the cytoplasm. These are the vacuoles. As the cells grow older the vacuoles become larger and ultimately fuse into one large vacuole occupying the greater part of the cell cavity. Strands of cytoplasm traverse this vacuole and may even in the middle of the vacuole form a mass enclosing the nucleus. In the oldest cells which are still living even these strands may disappear and the cytoplasm is limited to a scarcely perceptible layer surrounding the vacuole. In this case the nucleus lies in this lining layer of cytoplasm; it is never in direct contact with the vacuole.

The contents of the vacuoles are very varied. Occasionally solid particles may occur, as for instance, those of calcium sulphate in certain Desmids. Such particles have been observed by G. S. West in a number of green algae (Price, 1914). Apart from these are particles of ultra-microscopic dimensions which have been observed by Price. Undoubtedly, however, as the facts of osmotic pressure and turgor show, the contents of the vacuole consist chiefly of an aqueous solution of various substances. Thus in the bulb of the onion and in the root of the beet the substance is mainly sugar (De Vries, 1884); in the bean, pea, buckwheat and maize it is said to be chiefly potassium nitrate (Copeland, 1897); in other plants

potassium chloride may be present in considerable quantity, while in many cases organic acids are the principal substances present (De Vries, 1879, 1883; Kraus, 1886).

At the junction of the vacuole and the cytoplasm we have again a surface separating two immiscible phases with consequently properties differing from those of either phase, and it has become usual to regard that part of the cytoplasm directly surrounding the vacuole as a distinct layer spoken of as the internal plasmatic membrane (Pfeffer, 1900) or the vacuole wall (De Vries, 1884). Price, by ultra-microscopic observation, has obtained indications of a definite layer in this location similar to that bounding the cytoplasm externally. To this question we shall have to return later.

Yet another complication is present in the case of plant cells, namely, the cell wall. Although this is absent in a few cases, nearly every plant cell is bounded by a firm envelope which thus separates the individual protoplasts from one another. The cell wall differs widely in morphological construction and chemical composition among different species and in different parts of the same plant, but with the exception of the Fungi one of the main constituents of the cell wall is cellulose, a carbohydrate, or more probably a group of carbohydrates, of high molecular weight. A number of other substances are present, these varying in different species and in different tissues of the same species. In the Fungi, including the Bacteria, the principal constituent of the cell wall is chitin. The cell wall of the young plant is always thin, but after the cell has reached its ultimate size, various morphological and chemical changes take place in the cell wall, for a description of which reference may be made to standard botanical text-books (for example, Sachs, 1875; Strasburger, 1903) and especially to the work of Mangin (1893). It should be mentioned here that thickening of the cell wall may consist entirely of cellulose and allied substances, or the walls may be modified by the processes of lignification, suberisation or cutinisation, in which various substances grouped under the names lignin, suberin and cutin (see Priestley, 1921) are deposited in the cell wall and profoundly alter its properties. It should also be observed that when cell walls become thickened the thickening is rarely uniform. Scattered over the cell walls places occur at which thickening does not take place so that thin spots known as pits are dotted over the surface. The pits on the two sides of a wall separating two cells are always opposite one another, so that at the pit there is only the original primary wall separating the two protoplasts. Chiefly through the

researches of W. Gardiner (1884), A. Meyer (1896), Strasburger (1901) and A. W. Hill (1901) it is clear that fine cytoplasmic threads penetrate the pit membranes, and sometimes indeed the whole thickness of the cell wall, so that in spite of the presence of the cell wall there is actually a continuation of protoplasm throughout the plant. Cellulose walls readily absorb water, and in the living plant the cell wall is normally permeated with imbibed water. Cutinised and suberised walls are however more or less impermeable to water, and their principal function is to prevent loss of water from the surface of cells.

Such then is the system with which we are concerned in a consideration of the problems of permeability. This system is very varied, attaining its highest complexity in the adult plant cell. In the latter we have to recognise at least three phases, the cell wall, the protoplasm and the vacuole. Each of these moreover is itself a complex system, both the cell wall and protoplasm each containing a more watery phase and at least one other phase, while there is evidence that the cell sap in the vacuole may also contain a colloidal disperse phase as well as water with substances in pure solution. At the boundaries between outer medium and cell wall, cell wall and protoplasm, protoplasm and vacuole, there are separating layers which there is every reason to believe have different properties from the bulk of the phases they separate. Further, in both the cell wall and protoplasm, and perhaps also in the vacuole, we have at least two-phase and probably polyphase systems in which there are consequently relatively large surfaces of contact between the phases. The constituents of the different phases vary from plant to plant and from tissue to tissue in the same plant.

We thus see how much phase boundaries figure in the structure of the cell, and it is impossible to lay too much stress on the importance that surface phenomena must play in regard to cell permeability. Before passing on to problems of the cell itself it will therefore be necessary to discuss briefly the more important facts relating to surfaces, as well as other physical and physico-chemical principles with which acquaintance is necessary for a proper realisation of permeability phenomena.

(To be continued.)

STOMATA AND HYDATHODES IN *CAMPANULA ROTUNDIFOLIA* L., AND THEIR RELATION TO ENVIRONMENT

By MARGARET W. REA, B.Sc.

Queen's University, Belfast.

(With 6 figures in the text.)

MANY authors have commented on the varying number of stomata observed either on the leaves of related plants, or on the leaves of plants belonging to widely differing families. Very little work so far has been done, by using the same species grown under different conditions, to demonstrate the possibility that the number of stomata per unit area may vary according to the leaf surface and the position of the leaf on the shoot. In this connection Professor R. H. Yapp found in *Spiraea Ulmaria* L. (= *Ulmaria palustris* Moench.) a range from about 300 to nearly 1300 stomata per sq. mm. on the under surface (there were practically no stomata on the upper surface) for the lower and upper leaves respectively of the same shoot (7, p. 827). He states: "It may be that *Spiraea Ulmaria* is exceptional in the latter respect, but it would be worth while comparing the successive leaves of a number of other plants, for if the phenomenon is at all general, the numbers already published for a large number of species might need considerable revision."

HISTORY.

Weiss (6, pp. 166-167) was one of the earliest workers who compared the numbers of stomata occurring in a large series of different plants. He found that members of the same family or even members of the same genus may show great differences in the number of stomata present, although the habitat, temperature and amount of moisture in the soil is the same (6, pp. 166-167). His conclusion is that habitat has no influence on the occurrence of the stomata. The halophytes, such as *Arenaria grandiflora* (6, pp. 123-132) and *Chenopodium ambrosioides*, had more stomata on the upper than the

under surfaces. This was also true of Conifers and Monocotyledons such as *Asphodelus luteus* and *Iris germanica*.

Tschirch at a later date reviewed the work of the earlier writers and quotes Morren as considering that "in related plants the number of stomata was in simple relation to the need for water." Czech (5, p. 169) considered that plants, of the same organization and family with varying numbers of stomata, can close them at will and so guard against over transpiration. Zingeler (5, p. 169), who worked with the Carices, found that those growing in a dry habitat had few stomata while those growing in a damp habitat had many. Tschirch (5, p. 173) concluded that on the whole plants in dry, stony places had fewer stomata than those growing in fields. In both habitats there were species with more stomata per sq. mm. on the upper than the under surfaces.

	Upper surface	Under surface	
<i>Triticum sativum</i> ...	47	32	} Fields
<i>Secale cereale</i> ...	49	42	
<i>Avena sativa</i> ...	40	27	
<i>Sedum album</i> ...	49	25	} Dry, stony places
<i>Sedum acre</i> ...	21	14	

But *Triticum* and *Avena* have more or less isobilateral leaves while the species examined from dry, stony places were succulents. Miss Delf (2, p. 501) records that the number of stomata on *Salicornia annua* increased the higher the internode, and that their size varied with the age of the internode, being smaller on the upper leaves. From the fact that, although there were many stomata present in *Salicornia annua*, there were few in *Suaeda maritima* and *Arenaria peploides*, she concludes that "the distribution of stomata in halophytes is a variable feature and throws but little light on the problem of transpiration in these plants." Yapp (7, pp. 826-828) found many more stomata per unit area on the upper leaves of *Spiraea Ulmaria* than on the lower leaves. He concluded that the larger numbers on the upper leaves were counterbalanced by the smaller size of the stomata, the hairiness and the thicker cuticle of the leaves; and also that, considering the stomata alone, the transpiration from the smaller but more numerous stomata of the upper leaves is not much greater than that of the lower leaves with the fewer but larger stomata. Darwin (1, pp. 436-437) considered that his experiments indicated that transpiration is regulated by stomatal aperture, because on the whole there was a parallelism in the curves of transpiration and of stomatal condition.

Hydathodes are not considered in relation to the stomata in any of the above papers. It was hoped that a comparison of the leaves of shoots of *Campanula rotundifolia* grown under different conditions would add something to our present knowledge of the distribution and other characters of the stomata and hydathodes.

STOMATA.

This investigation was begun to see whether the number or other characters of the stomata, such as their arrangement and size, vary on the lower and upper surfaces of the same leaf, or on the different leaves of the same plant, or on the leaves of different plants grown under varying conditions.

Results.

In the method finally adopted for determining the total area of the leaf, each leaf was placed directly on squared mm. paper, its outline was drawn and the number of squares within the outline was counted. After being cleared in eau de Javelle and stained slightly with Bismarck brown, the total number of stomata for each surface was counted and from the data obtained the average number of stomata per sq. mm. was estimated¹.

Basal, intermediate and upper linear leaves were examined² (see fig. 1), and it was found that the number of stomata per sq. mm. for the under surfaces of each leaf was almost equal, the upper surfaces on the other hand showed a rapid rise from the lower to the upper leaf.

			Under surface	Upper surface
Upper leaf	120	67
Middle leaf	120	54
Lower leaf	112	14

"Whole" shoot³. From the lowest leaf upwards the under surfaces showed a fluctuating rise. The upper surfaces of the leaves showed a marked rise, especially near the apex of the shoot. The average for both was highest for the upper leaves (see Table I, p. 62).

¹ The midrib was included in the estimation of the area of the leaf. The inclusion of the midrib, on which some stomata were always present, makes the number of stomata per sq. mm. slightly less than would otherwise be the case. The smaller veins did not affect the distribution of the stomata.

² From a shoot gathered on Benevenagh Mountain, Co. Derry.

³ Grown in a garden, under normal conditions.

*Normal shoot*¹. Both surfaces showed a rise and fall in the number of stomata and it was noted that the stomata on the upper surfaces were nearly four times more numerous on the top leaf than on the bottom one (see Table II, p. 62).

*Shade shoot*². The under surfaces in this plant had a fairly uniform development of stomata from the base upwards. On the upper surfaces, however, the number varied to a greater extent (see Table III, p. 63). The number per sq. mm. was less on the whole than for the normal shoot (see Table II, p. 62).

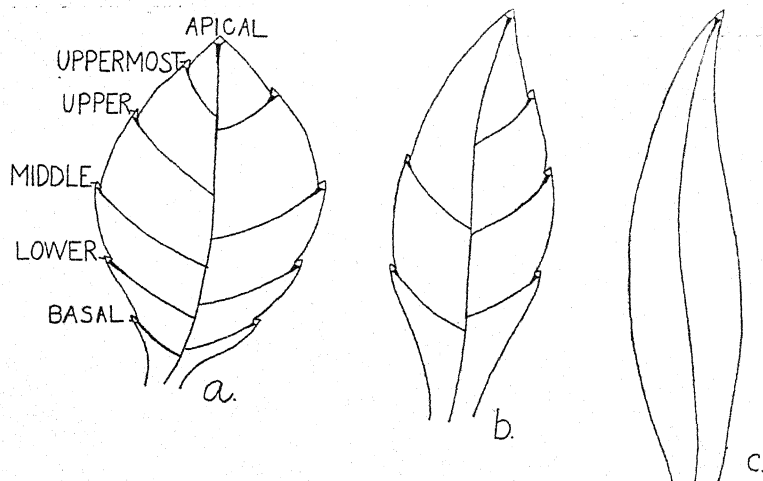


Fig. 1. Diagrams of (a) rotund, (b) intermediate, and (c) linear leaves, showing the position of the hydathodes.

*Sun shoot*³. This plant showed less fluctuation for the under surfaces, compared with the normal and shade shoots; with on the whole a higher number per sq. mm. On the upper surfaces of this plant for the first time, the numbers of stomata in some cases equalled or exceeded those obtained for the lower surfaces. The average number for both surfaces was much higher throughout the shoot than had been noted in the other plants examined (see Table IV, p. 63).

Arrangement of stomata. "*Whole*" shoot. The under surfaces of all the leaves had a row along the extreme margin, otherwise they were fairly evenly distributed. On the upper surfaces as a

¹ Grown in a garden, under normal conditions.

² Grown under a shady hedge, Newcastle, Co. Down.

³ Grown on a dry, sandy heath, Edzell, Forfarshire.

rule there were no stomata seen till about the fourth or fifth cell inwards from the margin.

Normal shoot. On the under surfaces there was no regular marginal row till the sixth leaf from the base. In the leaves higher up this row was sometimes present; while on the twentieth leaf it was noted that there was a marginal row along one side of the leaf and not on the other. The stomata were arranged evenly on the upper surfaces of all the leaves.

Shade shoot. On the under surfaces generally speaking, the stomata were more numerous. The stomata were evenly distributed on the upper surfaces.

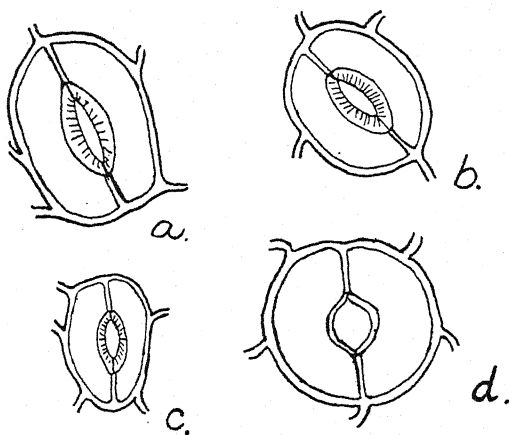


Fig. 2. (a) large, (b) medium, and (c) small stomata. (d) hydathode, from upper surface of a leaf. All $\times 500$.

Sun shoot. On the under surfaces there was usually no marginal row present. The twenty-sixth leaf had an irregular marginal row and the top leaf had two more or less regular rows round the edge. Otherwise in that region the stomata were arranged irregularly. On the upper surfaces they were again evenly arranged.

Size of stomata. The under surfaces of the "Whole," Normal and Shade shoots, showed a varying number of large, medium and small stomata (see fig. 2) the shade shoot having more medium sized pores. The medium size predominated on the under surfaces in the sun plant and on the upper surfaces of all three types.

Seedlings. The cotyledonary leaves of the outdoor and indoor seedlings examined had about the same number of stomata per sq. mm. on their lower surfaces; but on the upper surfaces the average was considerably higher for the indoor seedling. The average, however, for both surfaces of the two seedlings was quite comparable with that noted for the lowest leaf of the normal plant. It was remarked that the stomata were all large and well developed.

Outdoor seedling.					
Cotyledon	Area of leaf in sq. mm.	Lower surface		Upper surface	
		no. of stomata	av. no. per sq. mm.	no. of stomata	av. no. per sq. mm.
2nd	6.1	443	72.62	16	2.62
1st	5.5	411	80.9	18	3.27

Indoor seedling.					
2nd	6.8	463	68.38	28	4.12
1st	6.4	517	80.8	41	6.5

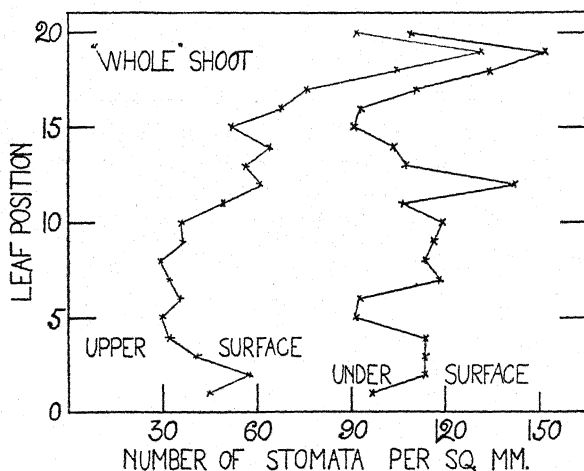


Fig. 3. Curves showing number of stomata per square mm. for upper and under surface of "whole" shoot according to position of leaf.

Curves were plotted, using the number of stomata per sq. mm. as abscissae and the position of the leaves, 1st, 2nd, etc., from the base upwards as the ordinates (see figs. 3-5).

Table I. Data concerning the stomata of the "Whole" Shoot.

Leaf position on shoot 1st = lowest 20th = highest	Leaf area in sq. mm.	Lower surface		Upper surface		Av. no. of stomata per sq. mm. for both surfaces
		No. of stomata	Av. no. per sq. mm.	No. of stomata	Av. no. per sq. mm.	
Linear	20th	1,107	108.53	940	92.16	100.34
	19th	2,888	152.0	2,498	131.47	141.73
	18th	5,488	133.85	4,294	104.73	119.79
	17th	5,366	110.64	3,495	75.81	93.22
	16th	4,554	92.0	3,400	68.69	75.34
	15th	5,039	90.30	2,931	52.53	71.41
	14th	5,072	103.09	3,130	63.62	83.35
	13th	6,281	109.29	2,275	56.47	82.38
	12th	7,893	141.45	3,392	60.79	101.12
	11th	6,297	106.19	2,948	49.71	77.95
Intermediate	10th	8,129	119.2	2,461	36.03	77.52
	9th	7,865	115.66	2,479	36.45	76.05
	8th	7,724	113.59	2,016	29.65	71.62
	7th	7,778	119.29	2,050	31.44	75.36
	6th	4,015	92.51	1,429	32.92	62.71
Rotund	5th	3,005	91.06	987	29.91	60.63
	4th	3,359	113.48	962	32.50	77.99
	3rd	2,738	113.61	997	41.37	77.49
	2nd	1,666	113.33	848	57.69	85.51
	1st	1,250	96.15	579	44.54	70.34
Total = 97,514				44,111		

Table II. Data concerning the stomata of the Normal Shoot.

Linear	24th	1.3	136	104.61	109	83.84	99.22
	23rd	4.4	300	62.12	235	53.41	57.76
	22nd	13.0	961	74.0	580	44.63	59.31
	21st	18.2	1,897	104.23	875	48.0	76.11
	20th	40.0	3,238	80.95	1,465	36.62	58.78
	19th	49.2	3,721	75.3	1,409	28.64	51.97
	18th	52.6	4,425	84.12	1,579	30.0	57.06
	17th	7.5	816	108.0	367	49.0	78.5
	16th	45.0	4,169	92.64	1,728	38.4	65.52
	15th	48.3	4,827	99.94	1,580	32.71	66.32
Intermediate	14th	36.2	3,232	89.3	1,223	33.8	61.55
	13th	33.3	2,484	74.6	1,164	35.0	54.8
	12th	38.3	2,496	65.17	1,284	33.53	49.35
	11th	34.2	2,348	68.65	1,264	37.0	52.82
	10th	38.2	3,308	86.6	1,923	50.34	68.47
Rotund	9th	40.2	3,800	94.53	2,028	50.45	72.49
	8th		8th leaf was missing				
	7th	28.7	4,950	172.82	2,390	83.28	128.05
	6th	40.4	5,970	147.77	2,153	53.3	100.53
	5th	24.3	2,331	96.0	863	35.52	65.76
	4th	37.4	3,497	93.5	1,110	29.7	61.6
	3rd	47.7	3,410	71.49	1,207	25.3	48.35
	2nd	48.0	3,225	67.19	921	19.19	43.19
	1st	15.8	886	55.54	385	24.36	39.40
Total = 66,427				23,842			

Stomata and Hydathodes in *Campanula rotundifolia* L. 63

Table III. Data concerning the stomata of the Shade Shoot.

Leaf position on shoot 1st = lowest 15th = highest	Leaf area in sq. mm.	Lower surface		Upper surface		Av. no. of stomata per sq. mm. for both surfaces
		No. of stomata	Av. no. per sq. mm.	No. of stomata	Av. no. per sq. mm.	
Intern.	15th	3,600	115.38	947	30.35	72.86
	14th	42.44	5,000	1,926	24.2	71.06
	13th	37.0	4,467	1,243	33.6	77.16
	12th	41.4	3,920	1,129	27.27	60.47
	11th	42.5	4,132	827	19.46	58.34
Rotund	10th	28.2	2,409	682	24.18	54.80
	9th	26.8	2,144	569	21.23	50.61
	8th	16.6	1,456	474	28.55	58.12
	7th	13.1	900	443	33.81	51.25
	6th	10.4	1,010	558	53.65	75.38
	5th	12.0	744	448	48.17	50.18
	4th	17.9	1,906	989	58.18	85.15
	3rd	17.4	1,974	782	44.94	79.19
	2nd	13.8	1,660	333	24.13	72.56
	1st	23.8	2,400	574	24.12	62.06
Total =		37,722		11,024		

The few upper leaves are not included in the table, as many of the stomata were immature (see fig. 1).

Table IV. Data concerning the stomata of the Sun Shoot.

Linear	40th	12.2	1,414	115.98	1,125	92.21	104.0
	39th	10.2	944	92.55	948	92.94	92.74
	38th	4.4	441	100.0	448	110.0	105.0
	37th	12.2	1,044	85.57	967	79.26	82.41
	36th	6.0	568	94.67	480	80.0	87.33
	35th	11.0	883	80.27	777	70.64	70.45
	34th	12.0	1,194	99.5	1,110	92.5	96.0
	33rd	11.0	1,045	95.0	802	72.91	83.95
	32nd	12.8	1,322	103.28	1,138	88.9	96.09
	31st	13.0	1,334	102.61	1,055	81.15	91.88
	30th	14.0	1,270	90.71	1,213	86.64	88.67
	29th	12.3	1,332	108.3	1,078	87.64	97.97
	28th	4.5	406	90.22	333	74.0	82.0
	27th	17.2	1,550	90.11	1,213	70.52	80.31
Intern.	26th	15.5	1,822	111.1	1,205	77.74	94.42
	25th	24.3	2,509	103.25	1,626	66.91	85.08
	24th	25.2	2,660	105.55	1,404	55.71	80.63
	23rd	29.0	2,310	79.65	1,646	56.74	68.19
	22nd	16.6	1,279	77.05	911	54.88	65.96
	21st	21.4	2,003	93.6	1,669	78.0	85.8
	20th	19.2	2,153	112.24	1,553	79.84	96.04
	19th	22.4	2,059	91.92	1,680	75.0	83.46
	18th	33.3	2,912	86.46	2,087	62.67	74.56
	17th	25.6	2,823	110.27	1,895	74.02	92.14
	16th	26.0	2,407	92.58	1,811	69.65	81.11
	15th	18.0	2,050	113.89	1,320	73.33	93.61
Total =		41,734		31,514			

The fourteen lower leaves had fallen off (see fig. 1).

Conclusions.

The average number of stomata per sq. mm. on the upper surfaces shows a steady rise from normal to sunny conditions (see Tables II to IV, pp. 62 and 63). This is not the case for the under surfaces, a probable reason being that the lower surfaces of leaves are always more or less in shade independent of habitat. The stomatal curves (see figs. 3-5) for the normal and shade shoots overlap: this is possibly due to the fact that the lower leaves of the normal shoot were shaded by other shoots.

The difference in the total number of stomata for the under and upper surfaces respectively of the "whole," normal, shade and sun shoots is instructive (see Tables I, II, III and IV, pp. 62 and 63). The normal shoot has about three times as many stomata on the under surfaces as on the upper; the shade shoot has more than three times the number on the under surface as compared with the upper, while for the sun plant the numbers approximate much more nearly for both surfaces. The "whole" shoot was grown under normal conditions, but the early summer of 1917 was drier and warmer than that of 1920 and this would account for the higher average number of stomata per sq. mm. compared with the number for the normal shoot examined in 1920 (see Tables I and II, p. 62).

The average number of stomata per sq. mm. for the under and upper surfaces of all the leaves of the shoots taken together was as follows:

		Under surface	Upper surface
Sun shoot	...	97.21	73.4
"Whole" shoot	...	111.93	50.63
Normal	„	89.5	32.12
Shade	„	100.96	29.5

It is evident that numerically the sun plant has more stomata per unit area than the other shoots, especially on the upper surface; and suggestions are put forward later to account for this fact.

Professor Yapp's work showed that the stomata increased in number on the higher leaves of *Spiraea Ulmaria* and did so more markedly on the plant growing in a sunny situation. Although the stomata occurred only on the under sides of the leaves, those data have more bearing on *Campanula rotundifolia* than any of the other work cited. Contrary to Tschirch's results, *Campanula rotundifolia* has more stomata per unit area in the sunny exposed situation than in the normal or shade conditions. This latter point is clearly shown

by the curves (see figs. 4 and 5, p. 67). Therefore it is concluded that the position of the leaf on the shoot and the habitat may be factors in the number of stomata present. Also, considering the number of stomata alone, it looks as if there was more transpiration from the sun shoot than from either the normal or shade shoots.

Other factors, however, may serve to lessen the effect of the more numerous stomata of the sun shoot. Foremost is the size of the stomata and especially of their pores. The large stomata on the under surfaces of the lower leaves of the normal and shade plants were absent from the under surfaces of the sun shoot. In most instances where there was an exceptionally large number of stomata per sq. mm. it was noticed that the stomata were small and had small pores (cp. 7, pp. 827-828). Therefore the transpiration might be little more from those leaves with a large number of small stomata, than from those where the stomata were larger but fewer. It may seem peculiar that the sun plant should form so many stomata, since considering transpiration alone, it might be inferred that the plant would suffer by losing more water than it could well spare in such a situation. Possibly, however, the question of photosynthesis is of such importance that the plant responds specially to it owing to the favourable conditions, and counterbalances the stomatal increase by making use of other devices to lessen water-loss which will be mentioned later. Haberlandt (3, p. 473) suggests in this connection that under these circumstances a current of air travelling through the whole thickness of the leaf is useful from the point of view of gaseous exchange.

The arrangement of the stomata was the same in all the shoots for the upper surfaces of the leaves, therefore neither leaf position nor habitat appears to be important. The arrangement was variable near the margin for the under surfaces of the leaves, and it is suggested that this is due to leaf position and possibly to other factors. It was concluded that the isobilateral tendency of the upper leaves of the normal shoot, and especially those of the sun plant, might be a factor in the equalization of the stomata on both surfaces of these leaves. Further, the linear shape of the upper leaves and also their thicker cuticle, as well as the smaller size of the cells in the upper leaves, would tend to reduce transpiration.

Miss Tenopyr (4, p. 216) states that "in *Campanula rotundifolia* the broad radical leaves had epidermal cells of an average length of .04 mm. and breadth of .039 mm. Those of linear cauline leaves had cells .034 × .032 mm., and transitional leaves .038 × .034 mm.

This agreed with Sierp's similar observations in *Mirabilis jalapa*, *Nigella damascena* and *Pisum sativum*. The higher the leaf the smaller its cells." The results obtained in 1917 when examining the leaves of the Benevenagh plant were thus confirmed. These factors would probably counterbalance the effect of any stomatal increase on the upper leaves of the shoots. Conversely the shade plant had more rotund and thinner leaves than the normal shoot. The part that hydathodes play in connection with water-loss in the leaves of *Campanula rotundifolia* examined, will be considered in the next section.

HYDATHODES.

While the leaves of the various plants were being examined for stomatal distribution, it was thought that the number of hydathodes which occurred on their upper surfaces might show some relation to their position on the leaf, to the position of the leaf on the shoot and to the environment of the plant.

The position and number of the hydathode groups on each margin of the upper surfaces were noted as shown in fig. 1. The total number of pores per leaf was ascertained and the results are given in the Tables V to VII, pp. 68 and 69) and are shown in the graph (see fig. 6, p. 67).

The hydathodes were developed on the lower leaves on slight marginal projections at the club-shaped terminations of the principal veins and the midrib. Usually there were three groups of hydathode pores on each margin, occasionally there were four or even five, nearly opposite each other, besides the apical one (see fig. 1).

The number of pores in each group varied, being most numerous in the apical group with one or two exceptions; and decreasing from apex to base of each leaf. The total number of pores per leaf shows a decrease from base to apex of each shoot. In a few instances the basal hydathodic organs had only one or two pores. The intermediate type of leaf had fewer marginal groups and only an apical one was present on the upper linear leaves. The individual pores were observed to be larger and clearer on the shade plant than on the normal or the sun shoot. The three leaves had the following number of pores present; on the lower leaf 32 pores; on the middle leaf 20 pores; and on the upper leaf 7 pores.

On the "whole" shoot the marginal groups were irregular in distribution, two being the highest number observed on either

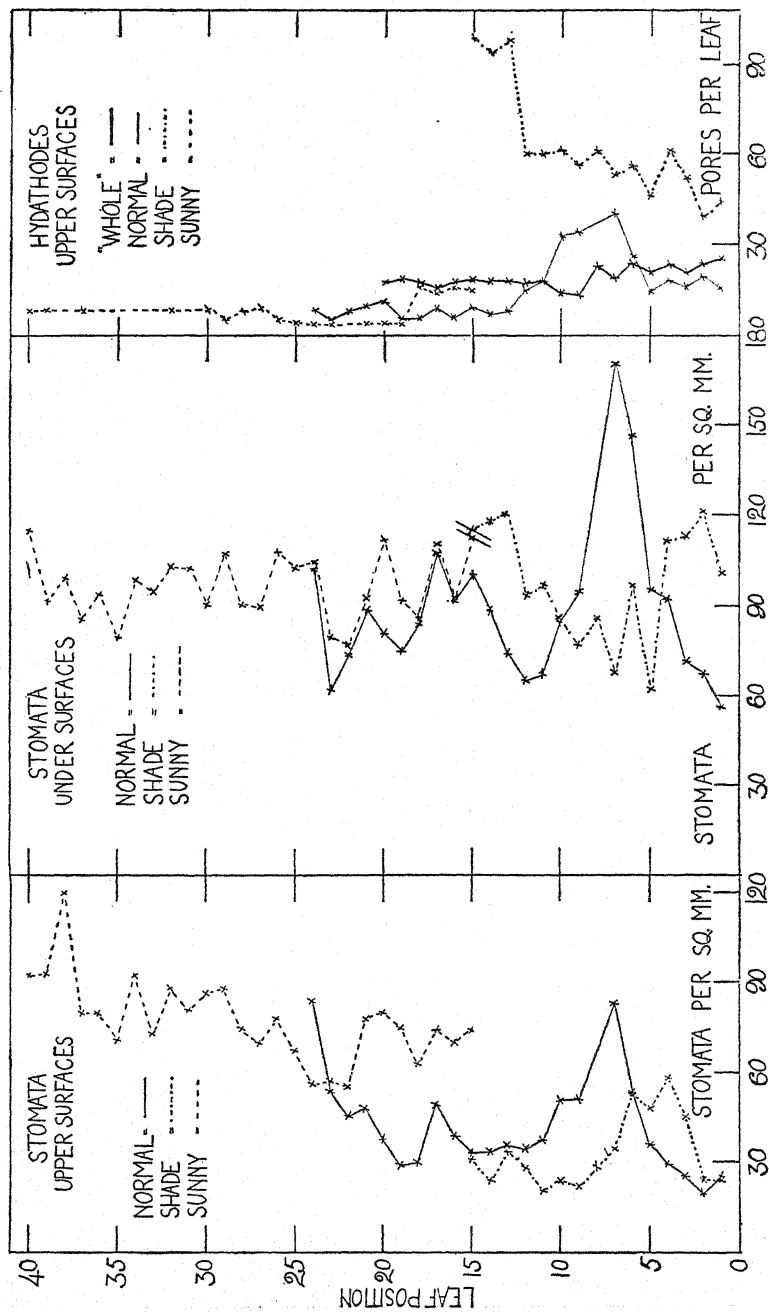


Table V. Data concerning the hydathodes of the "Whole" Shoot.

Leaf position on shoot 1st = lowest 20th = highest	Apical group	Groups right margin	No. of pores right margin	Groups left margin	No. of pores left margin	Total no. of pores per leaf
Intermediate Lin. { 20th to 9th	} 13 to 19	—	—	—	—	13 to 19
8th		—	—	1	middle = 5	23
7th		—	—	1	middle = 3	16
6th		2	middle = 4 lower = 2	1	middle = 3	24
5th	12?	1	middle = 4	2	middle = 3 lower = 2	21?
4th	11	2	middle = 4 lower = 2	2	middle = 4 lower = 2	23
Rotund { 3rd	14	1	middle = 2	1	middle = 4	20
2nd	13	2	middle = 3 lower = 2	2	middle = 3 lower = 2	23
1st	14	2	middle = 2	2	middle = 4	25
Total =						381

Table VI. Data concerning the hydathodes of the Normal Shoot.

Intermediate Lin. { 24th to 13th	} 5 to 11	—	—	—	—	5 to 11
12th		13	—	1	middle = 2	15
11th		9	2	2	upper = 1 middle = 1	18
10th		10	3	3	upper = 3 middle = 5 lower = 5	35
9th	8	3	upper = 6 middle = 4 lower = 4	3	upper = 6 middle = 4 lower = 2	34
8th	This leaf was missing	10	3	3	upper = 6 middle = 6 lower = 4	40
7th						
6th	6	3	upper = 4 middle = 5 lower = 3	3	upper = 2 middle = 2 lower = 4	26
5th	5?	2	upper = 1 middle = 2 lower = 3	2	upper = 4 middle = 2 lower = 5	14
4th	4?	2	middle = 3 lower = 3 upper = ?	2	middle = 5 lower = 2 upper = ?	18
3rd	4	2	lower = 3	3	middle = 3 lower = 1	16
2nd	4	2	—	3	upper = 4 middle = 4 lower = 1	19
1st	5?	2	middle = 4 lower = 2 upper = 4 middle = 4	1	middle = 3	15
Total =						345

The ? indicates that the number is correct to within one or two pores.

Stomata and Hydathodes in *Campanula rotundifolia* L. 69

Table VII. Data concerning the hydathodes of the Shade Shoot.

Leaf position on shoot 1st=lowest 15th=highest	Apical group	Groups right margin	No. of pores right margin	Groups left margin	No. of pores left margin	Total no. of pores per leaf
Intermediate	15th	4	upper = 15 middle = 9 lower = 6 basal = 5	5	upper = 8 most upper = 13 middle = 11 lower = 6 basal = 6	99
	14th	4	upper = 15 middle = 11 lower = 8 basal = 4	4	upper = 12 middle = 9 lower = 8 basal = 5	94
	13th	4	upper = 15 middle = 10 lower = 9 basal = 5	4	upper = 13 middle = 7 lower = 12 basal = 6	98
	12th	3	upper = 11 middle = 12 lower = 7	4	upper = 10 middle = 9 lower = 8 basal = 1	75
Rotund	11th	3	upper = 15 middle = 8 lower = 9	4	upper = 12 middle = 10 lower = 8 basal = 2	75
	10th	4	upper = 14 middle = 11 lower = 9 basal = 3	3	upper = 11 middle = 9 lower = 6	76
	9th	3	upper = 8 middle = 10 lower = 6	3	upper = 3 middle = 8 lower = 9	56
	8th	3	upper = 7 middle = 8 lower = 7	3	upper = 9 middle = 7 lower = 8	61
	7th	3	upper = 9 middle = 6 lower = 4	3	upper = 9 middle = 8 lower = 5	53
	6th	3	upper = 10 middle = 8 lower = 4	3	upper = 7 middle = 7 lower = 5	56
	5th	3	upper = 8? middle = 7? lower = 3?	3	upper = 7 middle = 5 lower = 4	46
	4th	3	upper = 9 middle = 8 lower = 6	3	upper = 10 middle = 8 lower = 6	61
	3rd	3	upper = 10 middle = 10 lower = 4	3	upper = 6 middle = 7 lower = 3	52
	2nd	3	upper = 9 middle = 5 lower = 3	2	upper = 9 middle = 5	39
	1st	2	— middle = 9 lower = 9	2	middle = 9 lower = 8	44
Total = 985						

The ? indicates that the number is correct to one or two pores (see fig. 1)
For data concerning the hydathodes of the Sun Shoot, see p. 70.

margin of the leaf; and marginal groups ceased altogether after the eighth leaf from the base. The apical hydathodic organs were large, especially towards the top.

The lowest leaf of the normal shoot had 15 pores and the number gradually increased till the seventh, which had a maximum of 40; then a fall occurred, at first gradual, but very marked in the case of the eleventh leaf where the number was 18, whereas the tenth had 33. From the thirteenth leaf to the top or twenty-fourth one, only an apical group was present, with a varying number of pores.

The shade plant showed a strong contrast to the normal one. Both the rotund and intermediate leaves had more marginal groups of hydathodes and the individual pores were larger. The lowest leaf had 44 pores and with a few exceptions these increased in number till on the fifteenth 99 were counted.

In the sun shoot the scars of the first fourteen leaves alone remained. From the fifteenth to the eighteenth leaf there was an apical group of 8 pores, and in addition the right margins had 1-2 groups, comprising 3-8 pores. From the nineteenth to the fortieth leaf only an apical group of 4-9 pores was present. The reduction in the number of pores in the sun plant is very noticeable compared with the number in the normal plant, and this difference is much more marked between the sun plant and the shade plant.

Total number of pores in the several shoots:

"Whole" = 381, Normal = 345, Shade = 985, Sun = 153

The cotyledonary leaves of the outdoor seedling had each an apical group of 4 pores, the corresponding leaves of the shade plant having each 5 at the apex.

CONCLUSIONS.

1. The position on the leaf.

The hydathodes invariably occur over the marginal terminations of veins, particularly the midrib. It may be concluded therefore that they occupy a definite position on the leaf in relation to the conducting system.

2. The position of the leaf on the shoot.

Three or occasionally four hydathode groups were developed on each margin of the basal leaves; they were fewer in number on leaves of intermediate type, and the upper linear leaves possessed only the apical group. Epidermal transpiration from the upper

linear leaves is probably reduced to a minimum as the cuticle is slightly thicker than in the intermediate or basal leaves. It is generally recognised that of two leaves of the same volume, if one is flat and thin it will lose more by evaporation than a narrow thicker one.

Thus towards the apex of the shoot the leaves show an approach to xeromorphy which includes the degree of development of the hydathodic organs. The number of hydathodes present bears a distinct relation to the position of the leaf on the shoot. The above conclusions were applicable in varying degrees to the three leaves, to the "whole" shoot and to the normal, sun and shade shoots. The cotyledonary leaves appear to be exceptional, as although they were basal in position, a single apical hydathode group was present.

3. The environment.

From the fact that the hydathodes on the shade plant had more numerous and larger pores for comparable leaves than either the normal or sun shoots, it would seem that environment is an important factor in the development of these water-secreting organs. As they were most numerous at the base of the shoots, these pores are probably very efficient in getting rid of superfluous water. The danger of the internal tissues being flooded, in the absence of accessory means of increasing the water-loss, would be most acute in the shade and normal plants. The lower leaves were not present in the sun shoot, but the above suggestion would hold good, although the difference between that shoot and the normal one is not so marked. In such, a danger of internal flooding in the leaf would not be so acute, and further, the presence of a larger number of stomata on the upper surfaces of the leaves of the sun plant is probably correlated with this decrease in water-secreting pores.

SUMMARY.

Stomata.

1. The number of stomata per sq. mm. varies in *Campanula rotundifolia*, increasing especially on the upper surface with a higher position of the leaf on the shoot; and on the under surface also with increase of illumination and dryness of the habitat of the plant.
2. The increase in number of stomata per sq. mm. in the sun shoot as compared with the normal shoot is suggested to be due to increased photosynthesis, as involving the utilisation of more

carbon dioxide; the water-loss at the same time being reduced by reduction in the number of hydathodes, and by the more nearly erect position of the leaf.

3. The arrangement of the stomata varied according to the leaf surface, there being sometimes a marginal row on the under surface which was wanting on the upper side; but this character is variable.

4. The stomata on the under surfaces varied markedly in size for the lower leaves of the normal and shade shoots, being large, medium or small; while on the upper surfaces in these shoots and on both sides of the leaves of the sun plant the medium size occurred.

Hydathodes.

1. Hydathode groups were present on the upper surfaces of all the leaves examined.

2. They were most numerous towards the bases of the shoots, and only an apical group was present on the upper linear leaves.

3. From the data obtained it is concluded that hydathodes are developed to a degree which is dependent upon the development of the vascular system of the individual leaves, upon the position of the leaves on the shoot, and also upon the habitat of the plant.

Acknowledgments are due to Professor R. H. Yapp, at whose suggestion the work was commenced, for material; to Mr B. M. Griffiths for advice; also to Professor J. Small for material and his assistance in the preparation of the paper for publication.

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THE HYDRION DIFFERENTIATION THEORY OF GEOTROPISM: A REPLY TO SOME CRITICISMS

By JAMES SMALL

HAVING read with interest a recent criticism¹ of the "creaming" part of the hydrion differentiation theory of geotropism, the writer feels indebted to Professor V. H. Blackman for affording him an opportunity of making clear certain points in the theory which seem obscure to several correspondents. The publication of this theory was really an honest attempt to provide a working hypothesis for the elucidation of the nature of geotropic response. The theory is therefore open to considerable amendment and even to complete rejection, if and when experimental data or other known facts which are of sufficient weight are brought forward. But so far as purely theoretical considerations are concerned there is certainly no need to stumble over a particle as large as 0.0002 mm. in radius.

Throughout his article Professor Blackman reasons from the *assumption* that "the protoplasmic [particles] in question are ultra-microscopic" (*op. cit.* p. 40). He even writes (*loc. cit.*): "As by the author's hypothesis the particles are ultra-microscopic," and as this idea seems to be shared by a few others, the writer apologises for any ambiguity which may have given rise to this misunderstanding. So far as he is aware the term "ultra-microscopic" does not occur in any of the papers previously published. The hypothesis as originally stated² is that: "The protoplasm was conceived to be an *emulsion* of protein or protein-lipoid particles in a colloidal protein medium."

Reference was also made to the Brownian movement of these particles and to the *suspensoid* structure of the plasma membrane. In an abstract³ reference was again made to the *emulsion structure*. The term "emulsion" rather than "colloidal solution" has been used always, as far as the writer is aware, because of the

¹ *New Phytologist*, 20, No. 1, p. 38. 1921.

² *New Phytologist*, 19, Nos. 3 and 4, p. 50, § 3. 1920.

³ *Linnean Society*, 18th March, 1920.

comparatively large size of the particles which are considered to act in the "creaming" process.

At the same time, even in colloidal solutions *microscopic* particles can be distinguished in some cases. Linder and Picton¹, for example, "recognised four kinds of sols: *a, visible in the microscope.*" Further Taylor (*op. cit.* p. 11) states that: "The upper limit [of colloidal heterogeneity] has been fairly definitely established, and lies *above the limit of microscopic visibility.*" He gives $10\ \mu$ as this upper limit; "particles between these limits ($10\ \mu$ and $0.1\ \mu$) are termed *microns* in Zsigmondy's nomenclature." According to a recent report² on Ultramicroscopy "the lower limit for the *micron* is conventionally fixed at $.2\ \mu$ and corresponds with the limit of microscopic visibility as determined by Johnston Stoney." However, in order to remove any further ambiguity, the size of the particles which are supposed to cream has been given definitely³ as "very small, almost ultramicroscopic⁴ (i.e. $.2\ \mu$ to $.8\ \mu$ radius), or quite ultramicroscopic (i.e. less than $.1\ \mu$ radius)." Brownian movement is shown by such particles, "at this stage [$0.1\ \mu$] the phenomenon known as the Brownian movement, just observable at about 10^{-3} cm. [= $10\ \mu$], is well developed⁵."

Keeping in mind that the "creaming" particles may be anything up to $0.8\ \mu$ in radius, we may be allowed to readjust the data given in the first section of Professor Blackman's article. As pointed out in that paper, Perrin found "that for gamboge particles of radius $0.21\ \mu$ the concentration was halved for each rise in height of $30\ \mu$." Since a meristem-cell in a root-tip averages $20\ \mu$ to $40\ \mu$ in diameter, this degree of difference in concentration would seem to satisfy the requirements of the "creaming" hypothesis.

One other point may be noted from § 1. It is stated that: "As is well known, there is no obvious settling of colloidal solutions." Taylor (*op. cit.* pp. 56-57) gives amongst "the methods which connect the size of the particle with other properties of the sol," "(3) the *velocity of sedimentation,*" and he also gives the Stokes' Law equation as the basis of this method. It is true he adds that: "It

¹ See *The Chemistry of Colloids*, by W. W. Taylor, p. 58. London. 1920.

² B.A. Rep., Cardiff, 1920. Third Report of the Committee on Colloid Chemistry, p. 32.

³ See *A Textbook of Botany*, J. Small, p. 347. Churchill. London. 1921.

⁴ Since Abbe placed the lower limit of microscopic visibility at $0.8\ \mu$ to $0.2\ \mu$ this phraseology is quite justified (see *An Introduction to the Physics and Chemistry of Colloids*, by E. Hatschek, p. 24. London. 1919).

⁵ Taylor, *op. cit.* p. 11.

is only applicable when the dispersity is low, for if $r = 10 \mu\mu$ [0.01μ] the time to fall 0.1 mm. [100μ] is 7 hours." In the above-mentioned report we also find under Stokes' Law that, using this equation, "we have a ready means of determining accurately their size from diameters of 1 mm. to and *beyond the microscopic limit,*" and again (*op. cit.* p. 36) that this "method cannot conveniently be used for *submicrons* of less than $20 \mu\mu$ [0.02μ] diameter." Also (*loc. cit.*) certain ultra-microscopes "when arranged horizontally are well suited for the purpose of determining the radius of *submicrons* from *their speed of settlement by the application of Stokes' Law.*" From these quotations it would appear that "settling" in colloidal solutions is not only well known, but is carefully measured by some of those who deal in a practical way with such material.

In the second section of his article Professor Blackman raises several points which are best considered *seriatim*. (1) The *time factor*, with particles of radius 0.2μ to 0.8μ , does not present serious difficulties (see below for calculations). (2) "Perrin . . . allowed three hours for the completion of the process." This statement is brought forward to support the view that particles even of 0.2μ radius would cream too slowly, but Professor Blackman on a later page emphasises the fact that stimulation continues for some time after its commencement. Now it is the *beginning* of stimulation which occurs soon after the organ is displaced, and therefore we should consider, not the time taken by the particles to reach a stable equilibrium (as they did in three hours with Perrin's gamboge), but rather the time which elapses before the redistribution of the particles becomes apparent. This time is indicated by Perrin, who states¹ that when the *uniform emulsion* is placed in a cell 100μ high "a few minutes suffice for the lower layers to become manifestly richer in granules than the upper layers." The resulting stimulation, if any, would, therefore, *begin in a few minutes and continue for about three hours*, thus satisfying the requirements both of rapid and of continued excitation (see also below). (3) Perrin's data with a medium of viscosity 125 times that of water are next quoted. From this one infers that the critic considers protoplasm to be of a similar viscosity, but on the following page he takes a viscosity twice that of water for the protoplasmic medium. This is a wide range, and more restricted estimates are fortunately available.

¹ *Brownian Movement and Molecular Reality*, by J. Perrin. Eng. trans. p. 41. London. 1910.

Ewart¹ states that "we have weighty reasons for considering the viscosity of the main bulk of the streaming protoplasm to be within the limits $\cdot 04$ to $\cdot 2$ at 18° C." Since Siefriz² has shown that the active protoplasm of young cells has a minimum viscosity (i.e. something *below* $\cdot 037$), it is quite reasonable to take Ewart's lower limit ($\cdot 04$) as the viscosity of the protoplasm in the meristem. (4) Professor Blackman next states that my conclusion that the "creaming" is governed by Stokes' Law is certainly mistaken. As pointed out above (p. 72) the application of Stokes' Law is commonly regarded as an accurate method of measuring the rate of fall of particles (even down to $\cdot 02 \mu$ diameter) which are heavier than the medium, and there is no reason to suppose that a density difference in the opposite sense renders futile the application of a general law like that of Stokes. Although the ultimate distribution of even very heavy particles is determined by the exponential "rarefaction law," the initial stages of fall must be governed by Stokes' Law. Perrin makes this point clear, when he states (*op. cit.* p. 35) that: "It is necessary to employ a *capillary* tube to avoid the convective movements" which occur in *wider* tubes; that in "a shallow cylindrical vessel about 100μ in height" (*op. cit.* p. 31), "if our kinetic theory is exact, this [uniform] distribution will change from the time the preparation is left at rest" (*op. cit.* p. 41); and that this change is obvious in a few minutes (see above). Perrin is even more explicit in the extrapolation from Stokes' Law which he gives, adding (*op. cit.* p. 40) that: "The preceding experiments show that this law is valid in the domain of microscopic quantities, and the verification pushed even to the threshold of ultramicroscopic magnitudes, scarcely leaves a doubt that the law may still be valid for the far smaller granules of ordinary colloids, or for the *large ions* found in gases." And further (*op. cit.* p. 76), "Now the reasoning of Einstein supposes the *law of Stokes* to be valid. It is therefore probable that this law, the exactitude of which I have proved directly as far as dimensions of the order of a tenth of a micron (No. 21), still remains exactly verified for large molecules, the diameter of which *does not reach the thousandth of a micron*. It will permit us presently to apply the law of Stokes with safety to the case of ions in movement through a gas."

Stokes found that his law is valid so long as the radius of the

¹ *On the Physics and Physiology of Protoplasmic Streaming in Plants*, by A. J. Ewart, p. 19. Oxford. 1903.

² *Botanical Gazette*, 70, p. 360, Nov. 1920.

particle is small compared with the *critical radius*, which is $\frac{\eta}{v\rho}$, where η = viscosity of medium, ρ = density of medium, and v = velocity of particle¹. Arnold found that the law was valid so long as the radius of the particle was less than $\cdot 6$ of the critical radius (*op. cit.* p. 96); while Millikan working with gases found that Stokes' Law becomes invalid only when the radius of the sphere is comparable to "the mean free path" of a gas molecule, and mentions (*op. cit.* p. 98) that the "holes" in the medium or the mean free paths are negligibly small "when the drop falls through a liquid."

It seems clear, therefore, that although the exponential "rarefaction law" governs the final state of equilibrium, Stokes' Law must govern the actual falling or "creaming" of the particles. We may have to apply a correction, as Perrin (*op. cit.* p. 34) points out, for the recoil of the particles as they accumulate at the bottom or the top of the cell, i.e. as they approach their exponential distribution, but this would be a correction for an aberration from the basal law (Stokes') governing all slow movements of spheres through a relatively viscous medium under a constant unidirectional force. Further, although the smaller "ultra-microscopic granules within the narrow confines of a cell only 0.05 mm. in height must always be little removed from their limiting distribution," the larger microscopic or almost ultramicroscopic particles in the cell will be far removed from their limiting distribution, even in a cell only 30 μ in height, and they will *begin* to become redistributed in a few minutes as observed for Perrin's gamboge particles.

As an example of how far such particles may be removed from their stable distribution it will be sufficient to point out that Perrin (*op. cit.* p. 43), with particles of about 0.3 μ radius, found a height of 30 μ sufficient to lower the concentration of the granules to one tenth of its value. In this case 10 μ , not "30 millimetres," in the cell were equivalent to 6 kilometres in the air.

As an example of what is supposed to occur, we will take milk, regarded as an ideal emulsion and containing fats as a disperse phase with a protein solution as the continuous phase. The density difference in an average good sample of milk is 0.17, which is smaller than the density difference in Perrin's experiments, as Professor Blackman suggests it would be in the cell. Viscosity we may

¹ See *The Electron*, by R. A. Millikan, p. 95. Chicago. 1917. With particles moving 1.55 μ in 7 minutes in a medium of density 1.03 as calculated below the critical radius is about one kilometre.

legitimately take as being $\cdot 04$ (see above, p. 76). Then applying Stokes' Law, we find the rate of creaming to be 1.55μ in the 7 minutes of Presentation Time¹ with particles of $\cdot 2 \mu$ radius, and 24.8μ in the same time with particles of $\cdot 8 \mu$ radius. Since the particles *begin* to cream almost immediately, when the organ is laid horizontally, a very short Perception Time can be detected by suitably delicate apparatus².

An interesting point is that, with short periods of intermittent stimulation on a klinostat, each particle would remain for some time during rotation very nearly³ at the point which it had reached during each successive stimulation; and no curvature would take place until the total potential difference produced by the "creaming" particles was sufficiently strong to develop in its turn a turgor difference (between upper and lower sides of the organ) which would be sufficient to overcome the mechanical rigidity of the organ. In this way we arrive at some sort of explanation of the occurrence of Presentation Time and Relaxation Time⁴. Therefore, whereas it may "seem impossible to correlate active electrical and mechanical reaction occurring in a few seconds or minutes with a redistribution of plasma particles requiring days for its accomplishment," when we assume that at least some of the particles are near but not under the limits of microscopic visibility, we can carry the correlation of the redistribution of such particles with geotropism into practically all the details of that most obscure physiological phenomenon.

In the third section of his article Professor Blackman raises another difficulty, namely the disappearance of potential differences when the "creaming" is completed. It is quite reasonable to suppose that protoplasm, like most colloidal solutions and emulsions, contains dispersed particles which vary considerably in size. The larger *microns* are supposed to bring about the rapid perception and the initial action current by their relatively quick "creaming." But Professor Blackman so ably expounds in the earlier sections of his paper the slow rate of creaming of the *smaller* particles, that it is scarcely necessary to point out that these *submicrons*, too, may be taken as electrically charged; and that by their *slow* creaming,

¹ For the broad-bean root. ² See *Proc. Roy. Soc. B.* 90, p. 351. 1918.

³ The "outward" movement according to Gibbs' Law being slower than the "creaming."

⁴ Would heavy particles which stick and then produce "a sudden precipitation of geo-electric response" show any definite Relaxation Time?

"requiring days for its accomplishment," these particles may *slowly* produce potential differences which would continue the action current¹ *initiated* by the "creaming" of the larger particles. Now, if we suppose a potential difference to be developed in each cell on displacement of the organ, we have the possibility of an action current for several days. Further, *only meristems are supposed to be geotropically perceptive*, at any rate in the higher plants. Cell division in meristems is frequent, and some cells are always in the process of division. During cell division there is naturally a more or less complete redistribution of the dispersed particles, and each daughter cell may be considered as showing "creaming" for at least several days after its formation. It will be remembered that, when the main apical meristem becomes inactive in any way, its functions are taken over by a lateral branch. Since the "normal polarity current" is dependent upon the meristematic activity and controls the angle of the lateral organs, the disappearance of that activity involves the disappearance of the control of the lateral organs, until one of these assumes the vertical position, and takes over control by producing a "normal polarity current" instead of an "action current."

Professor Blackman then proceeds to quote two experiments by Bose. These can be dealt with briefly. In the first there is no evidence that the electrode position in the endodermis was distinguished from a close approximation to the adjacent meristematic region of the cambium, which might have been the gravity-perceptive region in that case. The second is surely a special case, since Bose himself has recently² stated that "the electrical response is found to increase as the sine of the angle of inclination," and he is supported *on this point* by the observations of Fitting and the writer³. Since the phenomenon is exceptional, even in Bose's experiments, it follows that a special enquiry is necessary and a special explanation probable.

Professor Blackman supposes that my theory "rejects the movement of comparatively large cell particles (such as starch grains) as the first step" and quotes the experiments by Bose in support of the statolith hypothesis. The presence of statoliths and of gravity

¹ Possibly to a lesser degree, but the smaller movement might be compensated by the greater number of smaller particles and the resulting possible increase in total potential difference.

² *Nature*, 22nd July, 1905, p. 650.

³ See *Proc. Roy. Soc. B.* 90, pp. 349-360. 1918.

perception have not yet been connected logically as cause and effect; they are as yet only frequently concomitant circumstances. The presence of "creaming" bodies on the other hand can be connected causally with the series of changes which are supposed to result in geotropic curvature, and the granular appearance of cytoplasm is too well known to require any special demonstration of the presence of bodies which may "cream." Let any reader carefully compare the numbers of phenomena which find an explanation under the statolith hypothesis with those which find at least a possible and reasonable basis in the chemistry and physics of the cell under the hydron differentiation theory, and then say which theory is the more scientific. The nature of the geotropic response, if the statolith theory be accepted, is one of the most obscure of physiological phenomena.

On the other hand, the hydron differentiation of the expressed sap is an experimentally demonstrated *fact* in the case of many roots and many stem structures¹. Roots are well known to be acidic, beyond a P_H 4.5, and the single fact that chlorophyll is stable only in a relatively alkaline medium² makes the relative alkalinity of green stem structures an obvious fact, since there is not a more widespread indicator. It may be objected that the "reaction" of the cytoplasm has not been investigated, but there is no reason for supposing that it is differentiated in the *opposite* sense³.

If the propagation of the gravity stimulus is due to potential differences giving an electric current which modifies the permeability of the cells⁴, the direction of that current and of subsequent curvature is almost certainly determined by the movement of electrically

¹ See A. R. Haas in *Jour. Biol. Chem.* 27, p. 225, 1916; J. Hempel in *Compt. Rend. d. trav. d. Lab. d. Carlsberg*, 13, Liv. I, 1917; and H. Kappen in *Landw. Versuchst.* 91, p. 1, 1918 (Abst. in *Bot. Abst.* II, 4, p. 143. 1918).

² Cf. *Practical Plant Biochemistry*, by M. W. Onslow, pp. 37-38. Cambridge. 1920.

³ Cf. A. R. Haas in *Bot. Gaz.* 63, p. 232. 1917.

⁴ That it is permeability which acts in producing the turgor differences seems to be the only alternative left since the viscosity differences recorded by Weber (*Oesterr. Bot. Zeitschr.* 64, p. 439. 1914, also *Jahrb. f. wiss. Bot.* 57. 1917) have been shown by Zollikofer (*Ber. D. Bot. Gesell.* 35, p. 291. 1917) to be founded on faulty experimental methods, while Phillips (*Bot. Gaz.* 69, p. 168. 1920) has shown fairly conclusively that no differences occur in the two sides of the stimulated organ, either in water content, or titration acidity, or hydron concentration, or catalase activity, or sugar content, or percentage of nitrogenous substances.

charged particles which become redistributed when the organ is displaced. Then, if the obvious is admitted, and the root is taken as acidic and the stem as relatively alkaline, these particles must move *upwards* in both organs, not downwards; otherwise the current would pass in the other direction both in stem and root, and the stem would *normally* grow down, as it does practically every day in our laboratory *under the reversing action of excess of carbon dioxide*¹. If, however, it can be shown that starch grains or other heavy bodies in the cell aid in the upward movement of these other particles, or that such starch grains are *electro-positive* in *alkaline* media and *electro-negative* in acid media, then they may be correlated with geotropism, but this type of "amphoteric" electrolyte is as yet unknown to science.

As a contrast it is quite a logical deduction from the well-known influence of hydrion concentration on enzymic and other biochemical reactions², that the P_H of the protoplasm is important in cell processes. Haas³, for example, writes: "The reaction of protoplasm is one of the most important factors of metabolism." But the further deduction that this hydrion differentiation is the cause of the difference in geotropic response in stem and root is admittedly only a very plausible theory; nevertheless the experimental evidence in its favour rapidly accumulates.

In concluding it must be pointed out that all the steps which are supposed to occur in geotropic response are capable of experimental demonstration, if they do occur, and it is this aspect of the theory and its applications which is now receiving attention. I would also like to express my gratitude to Professor V. H. Blackman for the kindly and helpful interest he has taken in the theory and for giving me this opportunity of dilating upon certain aspects of it which, like other points, received only brief notice in the original condensed account.

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¹ See a forthcoming communication by Miss M. J. Lynn.

² See *The Determination of Hydrogen Ions* by W. M. Clarke, Baltimore, 1920; also I. Aggazzotti in *Archiv f. Entwicklungsmechanik*, 37, p. 1. 1913; W. J. Crozier in *Jour. Gen. Physiol.* 1, p. 581. 1919; and E. O. Schley in *Bot. Gaz.* 56, p. 480. 1913.

³ *Bot. Gaz.* 63, p. 232. 1917.

THE GROUPING OF VASCULAR PLANTS

By MARGARET BENSON

IN 1908 the writer reviewed in this journal (7, p. 143) the range of the sporangial (especially the microsporangial) apparatus of Vascular Plants. The suggestion was made that all the types borne on the sporophyte could be homologized and that, by accentuating the resemblances between synangia, sori, sporangiophores, half anthers and seeds, we should be better able to concentrate attention on their probable origin.

As it has been found impossible to extend the denotation of the term "sporangiphore" to cover so many differentiated types it will be clearer to employ a new term and that of "soroma" is now suggested. By "soroma" we mean the sporangial apparatus of the vascular plant *plus* the receptacle or stalk. The soroma of the Psilophytales already showed a considerable range of structure but it agreed in being borne on an axial-like arm of the thallus or plant body, and in being radially symmetrical. In the case of *Hornea*¹ the soroma shows a flattened apex and a central columella and on several occasions was found to branch.

These recent revelations as to the structure of the earliest known land plants have heightened the interest in the sporangiophore (soroma) as we can now interpret its stalk (receptacle) as homologous with the primal type of axis of radially symmetrical plants indistinguishable from Thallophyta. Such cases as *Sphenophyllum fertile* and the anomalous branching systems of the soromata (synangia) of *Tmesipteris* fall at once into line with the structures found in the Old Red Sandstone plants. Mrs Thoday's theory² of the axial nature of the pedicel of the so-called "sporophyll" of *Tmesipteris* is much strengthened and the two leaf-like lobes can now be safely interpreted as cladodified branches of this axis. Such primitive uninnervated leaves may be called "Haplophylls." In this discussion we will accept the view that Haplophylls are also characteristic of the great group of Lycopodiales although in some species of *Sigillaria* we find a variant.

¹ Kidston and Lang, *Trans. Roy. Soc. Edin.* 52, Part III, p. 614.

² Sykes, "Anatomy and Morphology of *Tmesipteris*." *Ann. Bot.* 22, 1908, p. 81.

In the Sphenopsida we find leaves which are also probably cladodified axes but they show a definite trend towards a repeated dichotomy of the veins and in many cases a palmate type of segmentation of the leaf. For such simply elaborated primitive leaves the term "Meiophylls" is suggested.

In the Meiophyll the branching is always a dichotomy in one plane so that the leaf is strap-shaped or flabelliform. In the interesting cases of vegetative branches of *Sphenophyllum* bearing both lacinate and entire Meiophylls, the entire are found to be lower down on the axis. Apparently the earliest record of the Meiophyll is to be seen in *Hyenia sphenophylloides* Nathorst (Middle Devonian)¹ where the appendages are either simple or dichotomously branched. Such appendages may have given rise equally to the segmented and the non-segmented cladodified leaves of the later species of *Sphenophyllum*. The same range occurs in the pinnæ of the early Fern Meriphyte such as *Sphenopteris* and it is obvious that the Meiophyll is homologous with such pinnæ. Though certain Fern pinnæ show this type of venation the complete leaf involves greater complexity.

Pseudobornia exhibits, so far as the writer knows, the most elaborated leaf of the Meiophyll type. It would be interesting to secure structural material of this variant.

In the "cone scale" of the Sphenopsid *Cheirostrobis* we find both soromata (sporangiophores) and a segmented bract-like structure. The dorsiventral branching of the so-called "cone scale" trace in the cortex of the axis is not to be regarded as a branching of the leaf trace, but the branching of the common axis trace which on dichotomy in the dorsiventral plane gives rise on the ventral surface to sporangiophores (axial structures) and on the dorsal surface to the Meiophyll which proceeds normally to branch in a palmate manner. Thus the very antiquity and hence primitiveness of this remarkable cone, so magnificently described by Scott, really is at the root of its complexity.

There is yet a third type of leaf among the Vascular Cryptogams, namely the complex meriphytic leaf of the Ferns. This type has been fully discussed by Lignier and others whose theories were critically examined and accepted by Tansley² in 1908. The extraordinary resemblance between what we regard as the leaf of

¹ Arber, *Devonian Floras*. Camb. Univ. Press, 1920. Fig. 25.

² Tansley, *Lectures on the Evolution of the Filicenean Vascular System*, 1908. *New Phytologist*, Reprint, p. 2.

Stauropteris (no stem has so far been discovered) and the fertile branching system of axes of some members of the Psilophytales seems to add a still fuller justification for the view that the "frond" of Ferns has been evolved from a branching thallus by the appendicularization of a system of branches whether fertile or barren. For such leaves the term "Meriphylls" is here proposed, as Jeffery's "Megaphyll" includes the Meiophyll.

The soromata were involved and with the new source of nutrition, which the cladodification of the system of axes supplied, are found to have themselves undergone elaboration into synangia and sori.

Segmentation of the synangium followed first into equivalent parts as in the *Simplices* and later along so many different lines of descent, as shown by Bower, into the *Mixtæ* type of sorus. In no case do we find an exception to the law that among the Meriphylls the soromata are "taken up" upon the leaves as the writer expressed it in the 1908 "Sporangiophore" paper. In contrast to this we note that among the Sphenopsida the soromata more or less retain their independence of the leaf even though the vascular supply may be given off from the axis together with that of the leaf as in the cone of *Calamostachys*.

Relative antiquity of strobilus formation.

Turning now to a consideration of the relative antiquity of strobilus formation in the three groups Haplophylls, Meiophylls and Meriphylls respectively, we see that strobilus formation occurred at a vastly earlier epoch in the Haplophylls and Meiophylls than in the Meriphylls. In fact it has never been recorded among the Filicales which are the only Cryptogamic representatives of the Meriphylls and was only attained relatively late by their seed-bearing congeners.

This fact may be correlated with the limitation of the branching of the originally appendicularized constituents forming the "cone scale"—only axes of the second or third order obtaining. In the Psilotales it is possible that the very frequent anomalies of *Tmesipteris* indicate that the telescoping down of the soromata-bearing axes to that now regarded as the normal may be comparatively modern. To sum up: in the character of the leaf, in the insertion of the soroma and in the date of strobilus formation we have characters distinguishing three groups of Vascular Cryptogams.

Let us now turn to the Spermophyta.

There is no group of seed plants with a universally uninerved leaf constructed on the plan of the Haplophylls.

A single vein occurs in some Conifers but the leaf trace is generally at least dual and leaves of different species of the same genus may have one vein or a trace that segments palmately as in species of *Podocarpus* and *Araucaria*. There is ample evidence that the leaf of the Cordaitales, Ginkgoales and Coniferae falls within the Meiophyll type.

In *Podocarpus* each of the pair of cotyledons has two bundles, and the generally accepted interpretation of polycotyledony in the Conifers is that it is due to segmentation of an original pair. Ample evidence of this is given in the papers by T. G. Hill and E. de Fraine, I to III "On the Seedling Structure of Gymnosperms¹." Not only the cotyledons of *Ginkgo* but all the foliage leaves are more or less lobed and this lobed, flabelliform leaf was especially characteristic of the ancient type of Ginkgoales called *Baiera*².

In the Cordaitalean circle of affinity there is no example known of a leaf with the veins branching otherwise than in the dichotomizing palmate method found in Meiophylls. It appears to the writer that such uniformity points to an important ancestral resemblance and probable affinity between the Sphenopsida and this ancient group of Families.

Let us turn now to the consideration of the insertion of the soroma and deal first with the megasoroma or seed. We find as pointed out in the discussion of the subject in the 1908 paper, "The Sporangiphore," we are possibly "dealing with plants which have never had their 'sporangiphores' taken up upon leaves and that, in fact, they are constructed in this respect upon the Equisetal plan where the sporangiphores are merely associated with bracts³."

This view has been adopted tentatively by Sahni in his recent review of the entire Gymnospermous series appended to his Monograph on *Acmopyle Pancheri* Pilger⁴. He shows there are two great divisions based primarily upon the manner in which the seed is borne, whether upon the leaf, or directly upon the axis. These two divisions he calls the Phyllosperms (leaf borne seeds) including the Cycadales and Pteridosperms, and the Stachyosperms (stem borne seeds) including the Cordaitales, Ginkgoales, Taxales and Coniferales.

¹ Hill and de Fraine, *Ann. of Bot.* 22, p. 689, and 23, pp. 189 and 433.

² See Seward's *Fossil Plants*, 4, pp. 1-60, where the Meiophyll type of leaf is well illustrated.

³ *Loc. cit.* p. 149.

⁴ Prof. Birbal Sahni, *Phil. Trans. Roy. Soc. Lond. Series B*, 210, pp. 299-302.

He thinks there is strong negative evidence against the view that a sporophyll of the megaphyllous type had been originally present and has been lost but he concludes that the meagre positive evidence at present available is distinctly in favour of a common megaphyllous (meriphyllous) origin for the Cordaitales and Pteridosperms. It is this view that the writer wishes to counter. It is to her incredible that such ancient plants as *Cordaïtes* could have retained a catkin-like brachyblast which in its entirety might be regarded as homologous with the meriphyll of a Zygopterid Fern and that the parts really represent obsolete meriphyles.

In turning to the insertion of the microsoroma or pollen-bearing apparatus we seem to have secured new light from the comparison of the so-called "stamen" of *Cordaïtes*, *Taxus*, etc., with the sporangial apparatus of the Psilophytales. In both, the soroma is radially symmetrical and terminal on a simple stalk-like body. Those of the older members of Ginkgoales appear to resemble *Cordaïtes*. We have instances carefully worked out of the abortion of certain sporangia of the terminal cluster in *Ginkgo*¹ giving the dorsiventral type of *Ginkgo biloba*. The same phenomenon occurs in the passage from the radial symmetry of *Taxus* to that of the dorsiventral symmetry of *Torreya*² and *Cephalotaxus*. Such changes prepare us for the type found in Araucariaceæ and the more or less peltate form found in *Juniperus* and other Conifers. The Psilophytales prepare us for a transition from a radially symmetrical axial structure to a lamella. It seems to the writer to be reading complexity into simplicity to fail to accept the microsoromata and the megasoromata (seeds) as inserted directly upon axes. If this is recognized to be the universal condition in Sahni's Stachyosperms to what does the condition point? The Stachyosperms are not members of the same series as the Ferns, nor of the Pteridosperms, Cycadophyta nor Angiosperms. They are Meiophylls and not Meriphylls—to use the nomenclature of the earlier part of this discussion. They probably have some kinship in the remote past with the Cryptogamic Meiophylls and their resemblances to Cycads in their seed must be due either to the seed having originated before the distinctive types of foliage leaf were differentiated or possibly are due to homoplasy.

Sahni³ and Sprecher⁴ have shown that the seed of *Ginkgo* has

¹ A. Starr, "The 'Microsporophylls' of *Ginkgo*," *Bot. Gazette*, p. 134. 1910.

² Coulter and Laud, "*Torreya taxifolia*," *Bot. Gazette*, p. 159. 1905.

close resemblance to the Cordaitalean and *Taxus* type, and possibly if we knew the history of the Cycad seed we could show that the resemblance to a Stachyosperm seed is the result of relatively recent convergence.

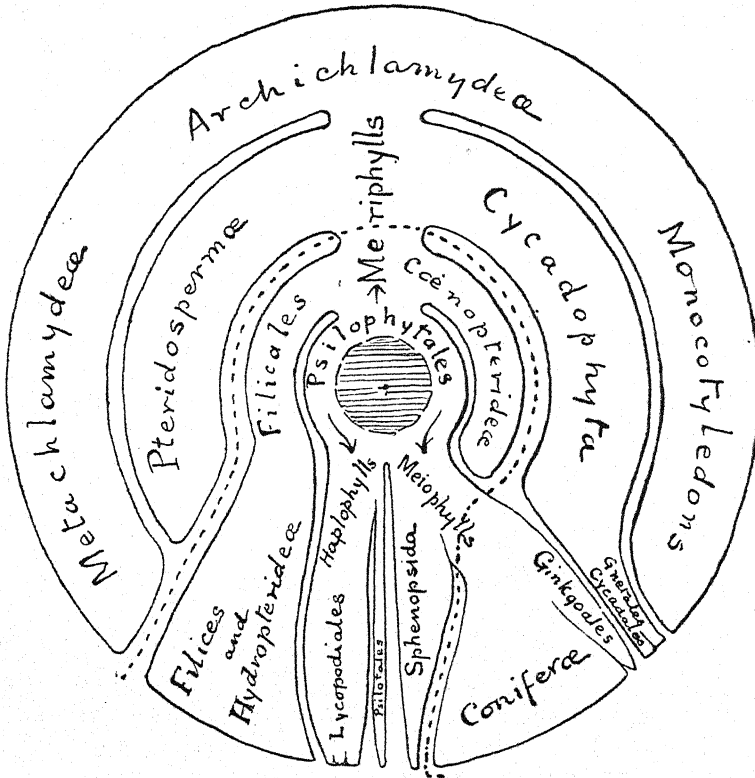
Further evidence in support of the original Meiophyll character of the leaves in Conifers is their early formation of a strobilus. The catkin of *Cordaites* is a strobilus and this strobilus or reproductive brachyblast (cf. *Taxus*) is regarded by the vast majority of morphologists at the present day as persisting in the form of the ovuliferous scale of the Abietineæ and in a more or less disguised form in the ligule of the "cone scale" of Araucarineæ where the one seeded character of the brachyblast is supposed to have facilitated a closer degree of fusion.

If one contrasts this early strobilus formation with that found among the Meriphylls where the earliest recorded case of strobilus formation is in the Triassic, we see how improbable as well as unnecessary it is to withhold credence from the view that the Stachyosperms are Meiophylls. It is for this reason they are entered as Meiophylls on the accompanying diagram on p. 89.

given to Prof. Seward's view if the Lycopodiales had not been regarded as the alternative to the Fern-Cycad alliance.

Finally the writer wishes to express her full appreciation of the difficulties of the problem. They have however been probably aggravated by a too facile acceptance of an affinity between Coniferæ and Gnetales. The latter group is regarded by the writer as a vestigial remainder of the Cycadophyta which instead of "magnifying the office" of the carpellary leaf (megasporophyll), as happened in Angiosperms, reduced it until it became obsolete. The Gnetales are probably a comparatively recent group, for there is no record of the Cycadophyta until the Triassic. As in the Cycadophyta we have in the Gnetales ample evidence of a micro-sporophyll and of a meriphyll type of foliage leaf, for both in *Gnetum* and *Welwitschia* we find reticulate venation and even a pinnate type in the cotyledon of the latter. On such grounds as these the Gnetales are entered in the accompanying diagram as surviving members of Cycadophyta.

A warning note may be given with respect to the diagram. It is not intended to denote the relative dominance of the different groups in past epochs for there can be little doubt that the Stachyosperms were more abundant in the mesozoic times than even the Cycadophyta. The diagram and indeed the whole of this discussion arose in connection with an attempt to discuss the possibility of securing a logical sequence of Families of Vascular Plants for practical purposes. (See the "Note on a Numerical Sequence of Plant Families," p. 90.)



DESCRIPTION OF THE DIAGRAM.

The periphery of the diagram represents the end members of three evolutionary series, the Haplophylls, the Meiophylls and the Meriphylls, and, very roughly, their relative dominance at the present time.

All Families that fail to reach the periphery are extinct.

All Families outside the dotted line are spermatophytes.

The circle in the centre, which is hatched, represents the pre-land-plants which, after Church, we are learning to call *Thalassiophyta*.

The annulus immediately surrounding this circle represents the earliest known vascular plants which may at present be identified with the *Psilophytales* of Kidston and Lang. From these proceed races with at least three types of appendicular, photosynthetic organs. It can be seen that the Haplophylls produced no seed plants; the Meiophylls are regarded as having given rise to the Sphenopsida, Cordaitales, Ginkgoales and Coniferae; the Meriphylls are the successful races, giving rise to the Ferns, the Cycadophyta, the Gnetales and the Angiosperms.

NOTE ON A NUMERICAL SEQUENCE OF PLANT FAMILIES

I N a recent number¹ of this journal a plea is put forward for a linear series of families of Vascular Plants. The fossil groups are not to be included, i.e. are not to have numbers assigned to them (*loc. cit.* p. 270) which is in harmony with the Botanical Garden outlook of the paper. Certain suggestions are made for the construction of such a series in a table, *loc. cit.* p. 269. As discussion is invited, I am venturing to make a few suggestions. A numerical sequence of families would certainly be useful from the point of view of economy of space in labelling museum specimens and living plants in a Botanical Garden. With a key to the family numbers, which Gundersen estimates at about 300, such labels, with the name of the species added, might be a great boon to students. Gundersen desires the sequence to be the result of general consideration "something like an inventory of facts which appear to have a bearing on family sequence." He is aware that the characters used in determining evolutionary sequence "are in nearly all cases very different from those by which plants are identified" and presumably would be prepared to have the Hydropterideæ broken up and one family associated with Schizæaceæ and the other with Hymenophyllaceæ.

But even then this ideal seems difficult to secure for a single linear series. If we omit all the fossil groups the linear series will run more or less at right angles to the radial series which indicate the evolutionary series.

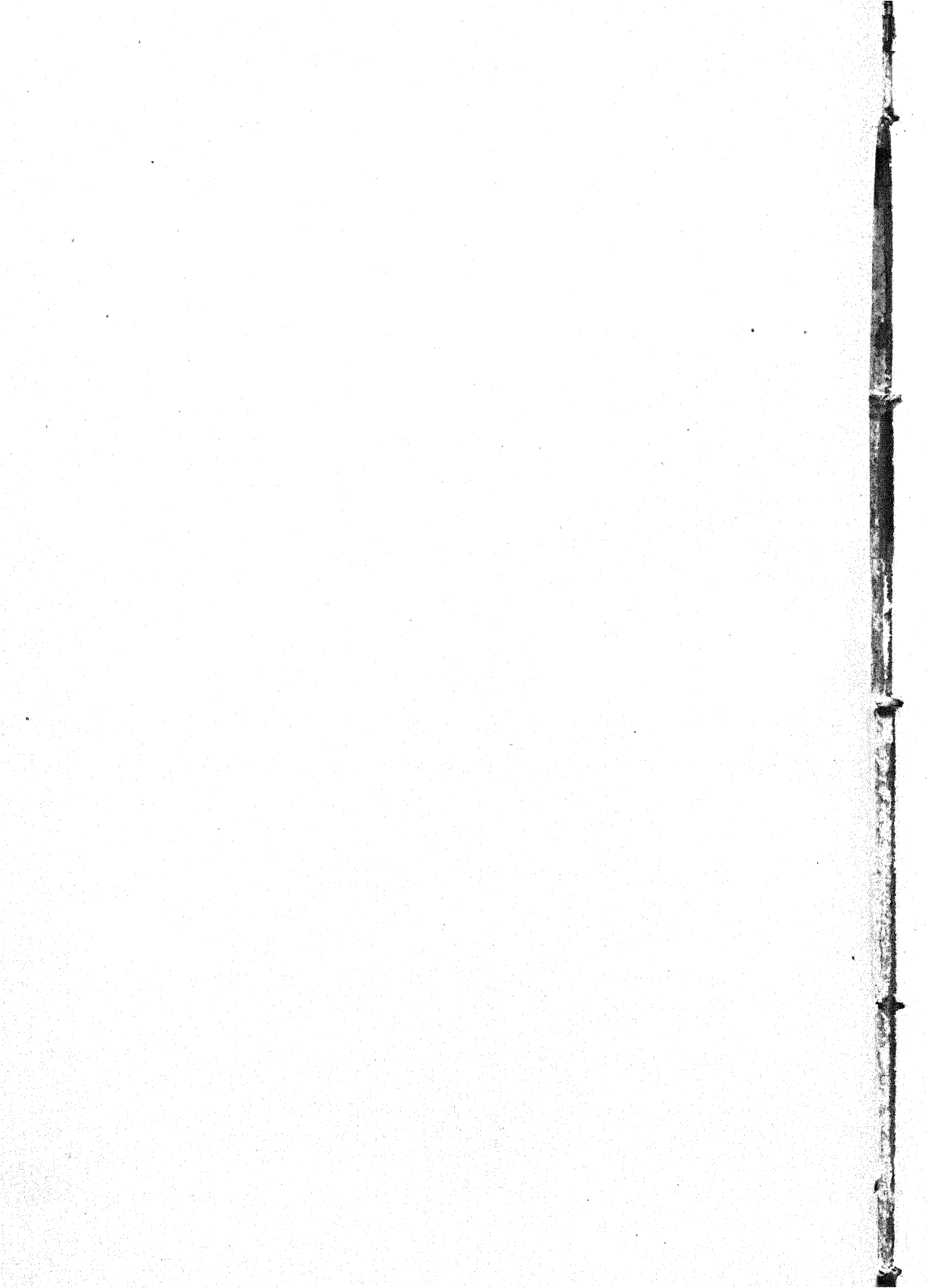
As, however, Gundersen obviously thinks the arrangement of the families in a numerical sequence should be on as natural a basis as possible I would suggest that it is of the first importance to determine the larger groups. In a paper in the current number of this journal I have discussed this question and have drawn up a diagram expressing the results to which I come. If this meets with his views let him record around the periphery of this diagram the 300 families to which he refers, locating them on that part of

¹ A. Gundersen, "Plant Families: A Plea for an International Sequence." *New Phyt.* 19 p. 264. 1920.

the periphery which is allocated to their corresponding major group. For Ferns I should advise a reference to Prof. Bower—for the Conifers there is at present very little consensus of opinion. The three families of Lycopodiales and one family each of Psilotales and Sphenopsida will offer no difficulty. It will be seen that if the sequence follows the periphery it will be only a formal list in which sometimes consecutive numbers are applied to nearly allied families and sometimes to families exceedingly remote from one another in affinity, as is the case when one passes from the Haplophylls to the Meiophylls, and thence to the Meriphylls. A reference must be made to the accompanying paper for definitions of these terms and for an explanation of the diagram.

If these suggestions are of little service, at least they will show how remote botanical problems (even the morphological) are from solution.

M. BENSON.



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PERMEABILITY

By WALTER STILES

CHAPTER III

SURFACE PHENOMENA

THE most obvious way in which the surface differs from the body of a liquid is in its behaviour as a thin stretched skin. This is most familiarly exemplified in the behaviour of films of soap solution.

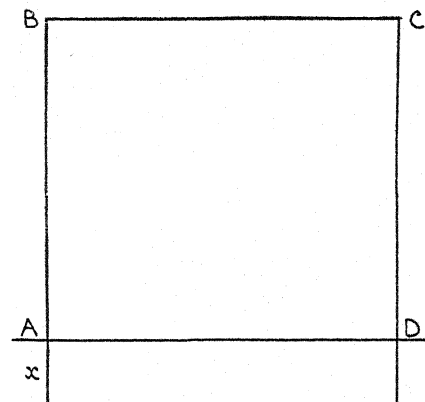


Fig. 1.

For instance if the rectangle enclosed by the wire frame *ABCD* (fig. 1) is occupied by a thin film of soap solution terminated at its lower edge by the wire *AD* which can move freely along the vertical wires *AB* and *DC*, the film will tend to contract and it is

necessary to attach a definite weight to AD in order to keep it in position.

The surface is thus the seat of special forces. How these come to be present can be understood from the following considerations:

In the mass of the liquid we have an enormous number of molecules, each one of which is surrounded on all sides by numbers of other similar molecules uniformly distributed round it. The mutual attraction of the molecules therefore results in nothing more than keeping them pressed together, the pressure on any particular molecule being uniform in all directions. By the force of cohesion the liquid particles are kept together, and do not tend to separate as in the case of a gas. At the surface of the liquid the state of affairs is different. A molecule at the very surface is subjected to the attractive force of molecules in the interior of the liquid, but there is no similar balancing force at the exterior. This means that the surface layer is subjected to an inwardly directed pressure at right angles to the surface, a pressure which decreases rapidly in the direction away from the surface as the cohesive force of the molecules is more uniform in all directions.

This inwardly directed pressure at the surface has two results. In the first place as all liquids are compressible to a small extent the surface layer of liquid will be compressed; in the second place as all the molecules at the surface are pressed towards the interior the surface will tend to contract to its smallest possible area.

So far the surface has been spoken of as if there were nothing outside the liquid. As a matter of fact there must always be some substance in contact with the liquid. Now a force of attraction called adhesion exists between molecules of different substances, consequently the actual inwardly directed pressure at the surface is the result of the difference between the pressure due to cohesion of the liquid and that due to adhesion between the liquid and the external medium. Only when the cohesion and adhesion are equal the pressure will be zero.

We thus see that the surface is in a state of tension by which it tends to reduce its area to the least possible. The *surface tension* of a liquid is defined as the force acting on unit length in the plane of the surface. From what has already been said it is clear that the surface tension depends not only on the nature of the liquid itself, but also on the medium with which it is in contact. The surface tension for instance of the surface water/air will not have the same value as the surface tension of the surface water/alcohol. When the

surface tension of a liquid is spoken of it is generally the surface tension of the liquid against air that is meant.

The surface tension of a liquid, besides depending on the nature of the liquid and on the medium with which it is in contact, also depends on the temperature.

It is outside the scope of this work to describe the different methods used for the measurement of surface tension. For these reference may be made to standard text-books of physics (*e.g.* Poynting and Thomson, 1905; Winkelmann, 1908). The principles of the more ordinary methods consist respectively of: (1) the measurement of the height to which liquid will rise in a capillary tube; (2) the measurement of bubbles and drops (Quincke); (3) the measurement of the size of drops of the liquid as it issues from a narrow tube; (4) measuring the least pressure necessary to force bubbles of air from the orifice of a narrow tube dipping in the liquid (Jaeger); (5) the determination of the deformation produced in the cross-section of a stream of liquid issuing from an elliptical orifice; (6) forcing a stream of the liquid upwards through a small orifice and measuring the height to which it will rise; (7) the determination of the velocity with which waves travel over the surface of a liquid (Rayleigh); (8) observations of oscillations of a spherical drop of liquid (Lenard, 1887). These and other methods will be found described in physical text-books.

The following table shows the surface tension of a number of liquids in contact with air, as determined by the capillary method, at 20° C.

TABLE I

Surface tension of certain liquids in contact with air
at a temperature of 20° C.

Substance	Surface tension in dynes per cm.
Water	72.53
Acetic acid	23.46
Ethyl alcohol	22.03
Ether	16.49
Chloroform	25.88
Olive oil	35.4

In Table II are given the surface tensions of a number of liquids against different substances.

TABLE II

Values of the surface tension of the same liquid against different substances at 20° C. (Data from Quincke)

		Surface tension in dynes per cm. against		
		air	water	mercury
Water	80.97	0	41.77
Mercury	53.98	41.77	0
Alcohol	25.49	—	39.93
Chloroform	30.61	29.52	39.93
Olive oil	36.88	20.56	33.54

The values given in this table are due to Quincke. His determinations are now generally regarded as somewhat high, and the figures given in Table I for surface tensions against air are lower in all cases than those found by Quincke. This does not affect the comparison of the surface tensions against different substances.

With increase in temperature the surface tension diminishes. Eötvös (1886) has propounded the following relation between surface tension and temperature

$$\frac{d(\sigma v^{\frac{2}{3}})}{d\theta} = -2.1$$

where σ is the surface tension, v the molecular volume (*i.e.* molecular weight/density) and θ the temperature whatever the value of θ and whatever the substance. From this equation can be calculated the value at which the surface tension becomes zero; calculation shows this temperature differs very little from the critical temperature. (Poynting and Thomson, 1905.)

Since the surface of a liquid is in a state of tension, it follows that when it contracts energy is released, and conversely, when a surface is increased work has to be done against the tension. Consequently the surface is the seat of energy. Referring to the example of the soap film in fig. 1, since the surface tension is the force exerted by unit length of the surface, and since there are two surfaces to the film, the weight required to keep the wire AD in equilibrium must be $2\sigma \cdot AD$.

Now if the film is stretched so that BA and CD are increased in length by a quantity x , the work done in stretching the film is

$$2\sigma \cdot AD \cdot x$$

or $2\sigma\omega$ where ω is the area by which the surface has been increased.

Consequently the potential energy of a surface is the product of the surface tension and the area. This quantity is called the *surface energy*.

It has been pointed out (Helm, 1887; Bayliss, 1915) that in general energy can be regarded as the product of two factors, an "intensity factor" of the nature of a force, and a "capacity factor" which is always a measure of size, such as mass, volume, area, length, etc. In the case of electrical energy for example, the intensity factor is difference of potential and the capacity factor strength of current. The capacity factors of two systems add together and the sum gives the capacity factor of the whole, the intensity factors cannot be added together. For instance in the case of heat, where the intensity factor is temperature, the sum of the temperatures does not give the temperature of the whole system. Differences in the intensity factor always tend to disappear. Thus two bodies in contact tend to come to the same temperature, or to the same potential. On the other hand there is as a rule no tendency for differences between capacity factors to disappear.

In the case of surface energy, the intensity factor is obviously the surface tension, and the capacity factor the area of the surface.

Now it is an important law of energetics that free energy always tends to reduce itself to a minimum, and hence the surface energy of a liquid will tend to diminish whenever this is possible. In most cases it is only the intensity factor which is capable of alteration to bring about reduction in the free energy, but in the case of surface energy both the intensity and capacity factors tend to diminish to the minimum possible value, that is the surface tension will reduce itself whenever possible and the surface will contract to the smallest possible. A reason for this has already been given in this chapter.

The tendency for surface tension to diminish is probably a principle of great importance in regard to permeability. In the case of two pure and immiscible liquids in contact there is no possibility for the surface tension to be reduced as this is a function of the nature of the liquids and the temperature. The case of a liquid that contains more than one component requires more consideration. In this case if the surface tensions of two components in the pure state are different, the surface tensions of mixtures of the two are intermediate between those of the pure liquids. Thus in the case of mixtures of ethyl alcohol and water, if the surface tension of pure water against air is taken as unity, that of ethyl alcohol against air is only 0.302. The curves in fig. 2 show how the addition of various alcohols to water lowers the surface tension of the latter. So far however it has not been possible to express the exact relationship

between surface tension and concentration of a solution algebraically.

In the case of a solution of ethyl alcohol in water the surface tension will obviously be reduced if the concentration of alcohol in the surface is raised. According to the principle first enunciated by Gibbs (1878, 1906) and emphasized by J. J. Thomson (1888), there will thus be a tendency for the alcohol molecules to migrate to the surface, so that the concentration in the surface layer is greater than

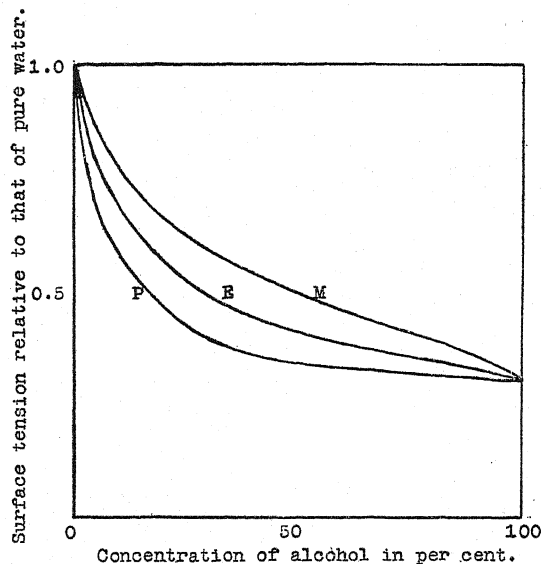


Fig. 2. Curves showing the relation between surface tension against air and composition of solutions of methyl (*M*), ethyl (*E*), and isopropyl (*P*) alcohols in water.

in the rest of the liquid. Opposing this tendency is that of the dissolved substance to diffuse from a place of higher to one of lower concentration. An equilibrium condition will thus be reached where these two opposed tendencies balance one another. It has been shown by Gibbs that when equilibrium is attained

$$\Gamma = - \frac{C}{RT} \cdot \frac{d\sigma}{dC}$$

where C is the concentration of the solute in the bulk of the solvent, Γ the excess of solute in the surface, $\frac{d\sigma}{dC}$ the rate of change of surface tension with concentration of the solute, T the absolute temperature, and R the gas constant.

The accumulation of a dissolved substance at the surface forming the junction between two phases is called *adsorption*, and when it takes place purely as a result of the mechanical surface tension it is called *mechanical adsorption*.

It will be observed that accumulation of solute in the surface layer can only take place if the dissolved substance lowers the surface tension of the solvent. If on the other hand the dissolved substance should bring about an increase of the surface tension, the concentration of the surface layer will be less than that of the rest of the solution, and negative adsorption will result.

Attempts to verify the formula of Gibbs have been made by Lewis (1909) and by Donnan and Barker (1911) and an approximate agreement with the formula has been obtained in some cases. In the following table are shown the values found experimentally for Γ compared with the values obtained by determination of $\frac{d\sigma}{dC}$ and subsequent calculation from Gibbs's equation.

TABLE III
Values of Γ found and calculated. (Lewis)

Substance	(In gm. per sq. cm.)	
	found	calculated
Sodium glycocholate	5×10^{-6}	7×10^{-8}
Congo red	3.7×10^{-6}	1.1×10^{-7}
Methyl orange	5.5×10^{-6}	1.2×10^{-7}
Sodium oleate	10^{-6}	10^{-8}
Sodium hydroxide	1.5×10^{-7}	7.5×10^{-9}
Caffeine	3.7×10^{-8}	2.4×10^{-8}
Sodium nitrate (kation)	2.5×10^{-8}	4.5×10^{-9}
Potassium chloride (kation)	5×10^{-8}	1.7×10^{-9}
„ „ (anion)	10^{-9}	1.6×10^{-9}
Barium chloride (anion)	not $> 10^{-8}$	1.6×10^{-9}
Copper chloride (anion)	3.5×10^{-8}	2×10^{-9}
„ „ (kation)	2×10^{-8}	2×10^{-9}

Lewis concludes that caffeine probably obeys Gibbs's Law quantitatively. In other cases the values found experimentally are always greater than those obtained by calculation with the exception of the anion of potassium chloride. Lewis thinks that some irreversible phenomenon such as gelatinisation at the surface may be the explanation of the discrepancy. As will be shown later in this chapter electrical phenomena are often present at a surface which add to the complexity of the matter.

Any substance dissolved in water lowers its surface tension against a solid or immiscible liquid, and this is usually the case also when

the solution is in contact with a gas. There are however some substances which increase the surface tension of water against air, among which are most inorganic salts, although these lower it at the interface between water and oil (Lewis, 1909).

An anomalous case of surface concentration is met with in the case of sugars. It appears that these substances in solution do not lower the surface tension of water. Nevertheless they are adsorbed at the surface to a slight extent (Adler and Hertzog, 1908; Hertzog, 1908; Rona and Michaelis, 1909). The explanation of this phenomenon offered by Rona and Michaelis is that the surface layer is in a state of compression (see p. 94) and the solubility of the dissolved substance is greater in this compressed layer than in the bulk of the solution.

In many cases the presence of the adsorbed material in the surface film of a liquid will increase the viscosity of the liquid at the interface. This may account for the formation of the rigid membranes observed by Ramsden. Similar membranes have also been obtained by Metcalf (1905) and Zangger (1908).

As we have seen in Chapter II we are concerned in the living cell with a decidedly viscous substance, and we can thus understand how the cytoplasmic "membrane" when damaged is immediately reformed, as has been often observed, *e.g.* by Chambers (1917) and Seifriz (1918).

Adsorption is in general a reversible process. In the case of such viscous fluids as solutions of proteins, soaps and bile acids, on the contrary, the process is irreversible.

Equations connecting the amount of adsorption at equilibrium with the concentration have been obtained empirically. Among the better known of these are those due to Küster (1894) and Schmidt (1894); the relation is most usually expressed in the form due to Freundlich (1909), which is

$$\frac{x}{m} = kC^{\frac{1}{p}}$$

where x is the mass of substance adsorbed by a mass m of the adsorbent, C is the concentration of the solution after adsorption has reached equilibrium, and k and p are constants. The value of p varies between 1.25 and 5, but it is very commonly about 2. This equation it will be observed may be written in the form

$$\log \left(\frac{x}{m} \right) = \frac{1}{p} \log C + K.$$

If then the logarithm of the amount of material adsorbed is plotted against the logarithm of the concentration at equilibrium, straight

lines will be obtained if the adsorption equation holds; this is shown to be approximately the case.

It follows from the adsorption equation that as the concentration of the solution increases the quantity adsorbed increases, but the quantity relative to the concentration decreases. For example, if finely divided charcoal is added to solutions of substances, adsorption generally takes place at the surface of the charcoal. Now if charcoal is added to a solution of acetic acid in water, when the concentration of the acetic acid at equilibrium is 0.018, the quantity adsorbed per unit mass of adsorbent is 0.467; when the equilibrium concentration is 2.79, the quantity adsorbed per unit mass of adsorbent is 3.76. That is, although $\frac{x}{m}$ in the adsorption equation has increased from 0.467 to 3.76, its value relative to the concentration of the solution, has decreased from 26 to 1.35.

As surface tension decreases with rise in temperature so adsorption also is less the higher the temperature. As the adsorption at a temperature $\theta + 10$ is thus a fraction of what it is at θ , adsorption has a *fractional temperature coefficient*, if the temperature coefficient of a process is regarded as the number by which the value of the process at one temperature has to be multiplied in order to give the value of the process at a temperature 10 centigrade degrees higher. The temperature coefficient is then generally denoted by the symbol Q_{10} . When the process, as in the case of adsorption, is lessened with rise of temperature, the temperature coefficient is less than unity. Such processes are often spoken of as having a *negative temperature coefficient*. This is not necessarily a misuse of the term negative, for by "temperature coefficient" is sometimes understood the quantity, or a multiple or fraction of a quantity, which has to be added to the value of a process for a rise of 1° C. This temperature coefficient is often denoted by the symbol α . It is unfortunate that the term "temperature coefficient" should be used in these two senses.

It must be noted that although adsorption decreases with rise of temperature, the rate at which adsorption is brought about increases. This is due to the fact that the rate of adsorption must depend on the rate at which the adsorbed substance can diffuse through the medium containing it. It is therefore to be expected that the rate of adsorption would have a temperature coefficient not far different from that for rate of diffusion, and this has been shown by Bayliss (1911) to be the case with adsorption of congo red by filter paper.

So far only pure substances, or solutions of one substance, have been dealt with. In the organism on the contrary we always have to deal with mixtures.

In general if there are two substances which can be adsorbed they displace one another to some extent. As the concentration of one of the substances increases, more of it is adsorbed and displaces the other to a greater extent, but relative to the concentration the amount of displacement decreases with increasing concentration. An exception to this is found in those viscous substances which tend to form rigid membranes at the surface. The presence of a viscous solute has no influence on the adsorption of a solute of low viscosity.

Another peculiarity of such substances is that the extent of adsorption depends not on their concentration, but on the absolute amount present. It is not clear how far the peculiar properties of such substances are due to high viscosity, low diffusivity or colloidal nature. Sugar as is well known considerably increases the viscosity of water, but the adsorption of sugar follows the ordinary laws and is reversible.

So far we have considered the consequences of the tendency of surface tension to reduce itself to a minimum. A few words are now necessary regarding the tendency of the area of the surface to diminish as far as possible. This is illustrated by the fact that liquids free from external forces always take up a spherical form, while if a liquid is divided into droplets scattered through another liquid of the same density, and with which the first liquid is immiscible, the droplets tend to unite into larger ones and finally into one single sphere.

In the case of living matter of a gel nature, however, we have a substance which possesses a certain amount of rigidity. Any change in form of droplets of such a substance will therefore be opposed by the resistance offered by the rigidity of the substance to change of shape, and a condition of equilibrium will be reached when these two tendencies are equal.

The tendency of surface tension in gels is therefore to bring about the coalescence of separated droplets. This union is called agglutination when microscopic particles coalesce into particles visible with the naked eye, and coagulation when the particles that unite are ultra-microscopic or very finely microscopic. Agglutination and coagulation may therefore be produced either by an increase in surface tension or by a decrease in rigidity of the dispersion medium.

So far we have considered the surface effects which are attributable to purely mechanical phenomena. The conditions at surfaces are however actually often more complex than has so far been indicated owing to the very general presence of electrical forces at the interface between two phases.

We know for instance that if a metal is immersed in a solution of one of its salts a difference of potential between the metal and solution results, and that the existence of this potential can be explained by the tendency of metallic ions to pass into solution. When this takes place the metallic ions give to the solution a positive charge, leaving the metal plate correspondingly negatively charged. The solution of the metal can therefore only proceed until the mutual attraction due to the difference in potential is balanced by the tendency of the metal to go into solution; *i.e.* by the electrolytic solution pressure of Ostwald which is proportional to the ratio between the concentrations of atoms in the metal and ions in the solution. The difference of potential is given by the expression

$$RT \log \frac{P}{p}$$

where P is the electrolytic solution pressure, p the osmotic pressure of the ions in solution, T the absolute temperature and R the gas constant.

The same considerations hold for hydrogen as for a metal and are the basis of the well-known electrometric method now so universally employed in physiology for measurement of hydrogen ion concentration.

In the case of the surface between a solid electrolyte and its solution similar considerations hold, though the matter is complicated by the presence of two ions. In this case the potential at the interface between the solid and liquid phases is given by

$$RT \log \frac{P^*}{p^*} \left[= RT \log \frac{p'}{P'} \right]$$

where P^* , P' are the electrolytic solution pressures of the kation and anion respectively and p^* , p' are the osmotic pressures of kation and anion in the liquid phase.

In general, for the potential difference due to one ion at the interface between any two immiscible phases, we have for the value of the potential the expression

$$\frac{RT}{nF} \log \frac{c_1}{c_2} + K$$

where c_1 and c_2 are the concentrations of the ion in the two respective phases, n the valency of the ion, F the electric charge of a monovalent gram-ion, and k a constant. It is outside the scope of this work to describe how these formulae are derived; those interested should consult the original work of Nernst (1889, 1892) and Haber (1908) and the general account of electrical phenomena at surfaces given by Michaelis (1914). For the application of these formulae to biological phenomena reference may be made to the papers of Beutner (1912, 1913) and Loeb and Beutner (1912).

It should be emphasized that the difference of potential (phase potential) arises from the unequal partition coefficients of the two ions between two phases, and is not connected with the different mobilities of the two ions. A difference of potential due to this latter cause (diffusion potential) arises when two solutions containing the same ions but in different concentrations come into contact. Diffusion takes place and if the mobilities of the two ions are different one ion will diffuse faster than the other and a difference of potential will thus result.

It appears however that the sign of the charge should be the same in whichever of the two ways the difference of potential arises, for in the latter case the solution takes the charge of the more mobile ion and in the former case it takes the charge of the more soluble one, and it appears that the more soluble ions are also the more mobile (Michaelis, 1914). Probably the electric charge at most surfaces can be accounted for in one of the preceding ways, but there are cases, as for instance that at the surface of aniline in contact with water (Ellis, 1912) where the aniline is negatively charged, although one would expect it to be positively charged as it feebly dissociates into the slightly mobile aniline ion $C_6H_5.NH_3$ and the very mobile hydroxyl ion. Although explanations have been offered of such cases (Lewis, 1910) the problem cannot be regarded as solved.

We may now pass on to a consideration of how adsorption is affected by electrical phenomena.

In the first instance we may consider a case of an electrolyte partly dissociated into its constituent ions. There will then be in solution the kation, the anion and the undissociated molecule all with their characteristic constants in regard to the adsorption equation, so that they tend to be adsorbed to different extents. Any difference in adsorption of the kation and anion must however be very slight as this would result in a potential difference between the surface and the interior of the liquid. An equilibrium position would

soon be reached where the force of attraction between the excess of oppositely charged ions would prevent any further separation. Such differences in potential due to inequalities of differently adsorbed ions are called *adsorption potentials* (see Freundlich, 1909).

The charge at a surface may itself be responsible for adsorption. By the principle that free energy will always reduce itself to a minimum, the electrical energy at a surface will tend to diminish whenever this is possible. Now if a surface is the seat of a negative charge, the deposition of any particle or ion carrying a positive charge will reduce the electrical energy of the surface, and such deposition will therefore tend to take place. That this is actually the case has been shown by Perrin (1904), Bayliss (1906), Lachs and Michaelis (1911) and others. For instance Bayliss has shown that a negatively charged surface such as that of filter paper will adsorb large quantities of an electropositive substance such as night blue, but only a trace of substance carrying a negative charge such as congo red. In some cases of such *electrical adsorption* the charge on the surface may actually be reversed; it seems likely that in these circumstances we are dealing with a complex effect in which both mechanical and electrical adsorption are involved.

Adsorption may obviously have an effect in regard to chemical reactions taking place at the surface. Thus if two substances which react together are adsorbed on the surface of a third, combination will take place on the surface of this third substance, which itself may remain unaltered. In a similar way reactions may take place if two substances mutually adsorb one another as in the case of barium hydroxide added to colloidal silica (van Bemmelen, 1910). A white substance is precipitated containing both substances, and from the mixture barium silicate slowly forms.

In such cases the active mass of reacting substances is the number of molecules adsorbed to the surface, and this number is proportional to the extent of the surface. Hence in such cases the rate of reaction is proportional to the surface. This is for example very generally the case in enzyme actions (see Bayliss, 1914).

Finally it must never be forgotten that all the phenomena of surfaces occur not only at the obvious surfaces of the cell, but throughout the colloidal substance of which the protoplasm is composed. Protoplasm is a heterogeneous system of more than one phase and throughout the system whenever there is a boundary surface between the disperse phase and the dispersion medium the surface phenomena described in this chapter must occur. A general

acquaintance is assumed here with the properties of colloids. Reference may be made to the works of Hatschek (1913), Taylor (1915), Bayliss (1915, 1918) and Wolfgang Ostwald (1909) in which colloids are treated from various standpoints.

OSMOTIC PRESSURE, ROOT PRESSURE, AND EXUDATION

By V. H. BLACKMAN

(With 3 figures in the text.)

THE exudation of sap from the cut stem of a rooted plant is such a striking phenomenon that it is not surprising that many attempts have been made to elucidate the physics of the process. A number of the older workers attempted to explain the phenomenon on simple physico-chemical principles and recently Rowell (1918), and Priestley (1920) in this Journal, have again discussed its mechanism.

It is usually assumed that the osmotic pressure of the living cells of the root and stem play a main part in the production of root pressure, and it is usually recognised that one of the chief difficulties is the passage of water or a weak solution from the living parenchyma cells into the cavities of the dead wood-elements. Priestley accordingly suggests that the explanation put forward by Lepeschkin (1906) of the exudation of water by the sporangiophore of *Pilobolus* and by the multicellular epidermal hydathodes of the leaves of *Phaseolus* and other plants may be used to account for the passage of water from the living cells into the dead wood-elements. Since Lepeschkin's views seem to have been accepted by some other botanists, and have received the support of Bayliss by inclusion in his *Principles of General Physiology* (1918), it would seem worth while to consider them in some detail.

Pfeffer (1877), at the time that he was making his classical researches on osmotic pressure, was the first to deal critically with the mechanism of the exudation of fluid from living cells. He formulated three hypotheses: (1) that the plasma membrane develops unequal osmotic pressures in different parts of the cell; (2) that there

is an unequal distribution of osmotic material in different parts of the cell; (3) that osmotic material is present in the cell wall outside the membrane so that water is sucked out of the cell. The first hypothesis is obviously unsatisfactory, since it makes osmotic pressure a function of the membrane instead of the concentration of the solution. At the time, however, that Pfeffer was writing osmotic pressure was very vaguely conceived, so that the presentation of such a view by a worker of the competence of Pfeffer is hardly surprising. In a later publication Pfeffer (1890) recognised the physical fallacy involved in this view, and characterised his first hypothesis as "irrig."

The value of Lepeschkin's work on *Pilobolus* and *Phaseolus* lies in the very careful measurements which he made of the effect of various factors on the rate of exudation of water. When, however, he deals with the interpretation of his results he passes in careful review the three hypotheses of Pfeffer outlined above and makes a surprising decision in favour of the first scheme—that of a cell with a plasma-membrane of different osmotic pressures in different parts, no reference being made to the fact that *Pfeffer had himself rejected this scheme fifteen years earlier.*

It is clear that the permeability of the membrane can only affect indirectly the osmotic pressure of the solution which it encloses by controlling through exosmosis the concentration of the solution. The osmotic pressure developed with such a leaky membrane will thus depend only in part on the specific permeability of the membrane. Other important factors will be the original concentration and mass of the solute and the time during which exosmosis has continued. If the membrane is rigid the pressure first developed will be practically the same as if the membranes were truly semi-permeable. Lepeschkin further holds that not only has a *membrane* a specific osmotic pressure, but that directly that pressure is exceeded water begins to pass out through the membrane (*loc. cit.* p. 425). The same solution can thus at the same time be both in equilibrium with and not in equilibrium with water; or rather it can be on both sides of the equilibrium point at the same time!

Enough has probably been said to indicate that Lepeschkin's view, that the exudation of fluid by *Pilobolus* and *Phaseolus* is due to the osmotic pressure of the excreting cell together with the differential permeability of the two sides of the cell, is supported by arguments of such doubtful validity that it cannot be considered acceptable. It is perhaps the barricade of mathematical equations

with which Lepeschkin surrounds his discussion of exudation that has protected him from the criticism that might be expected. The only other criticism that has been met with is that of Höfler (1920), who remarks in a footnote: "Lepeschkin's Ausführungen wonach der osmotische Druck der Zellen von deren Permeabilität abhängt scheint von theoretischen Standpunkt einer Revision bedurften."

Thoday (1918) has pointed out in this *Journal* how unsatisfactory is the usual presentation of the botanical aspects of osmotic pressure. For this the writers of text-books of physics and physical chemistry would seem in part responsible, for usually they do not sufficiently insist that osmotic pressure is the result of the tendency towards equilibrium between water and the solution, the absence of equilibrium being shown by the lower vapour tension of the solution as compared with that of water. The osmotic pressure of a solution is thus *an equilibrium pressure, i.e. the pressure which must be applied to the solution to bring it into equilibrium with water*¹.

To ascribe to the osmotic pressure of a given solution the responsibility for the exudation of water or of a weaker solution from that solution is certainly most unsatisfactory. Other pressures can be superimposed on osmotic pressure which may increase, decrease or even reverse it, but once osmotic pressure is recognised as an equilibrium pressure it is clear that it must always exhibit itself as a tendency to draw water into the solution, *i.e.* to bring about the absorption of water. Substances may pass out of the solution by diffusion if the membrane is permeable and so bring other forces into play outside the solution, or there may be other forces at work (such as electrical ones) which drive the solution out through the membrane, but such processes cannot be ascribed to the action of the osmotic pressure of the enclosed solution.

Since osmotic pressure is an equilibrium pressure it is obvious that by subjecting a solution to varying pressure it can be brought into equilibrium with—*i.e.* will neither give water to nor take water from—a solution of any concentration lower than that of the solution itself. It is easy then to appreciate that as a flaccid cell swells and the pressure of the cell wall increases the cell contents come into equilibrium with solutions of lower and lower concentration or osmotic pressure, and finally when the pressure exerted by the membrane is equal to the osmotic pressure of the cell contents the cell is in

¹ A very satisfactory treatment of osmotic pressure is that by E. W. Washburn, *An Introduction to the Principles of Physical Chemistry*. New York. 1915.

equilibrium with water. The osmotic pressure of the solution with which the cell is in equilibrium gives a measure—in terms of osmotic pressure—of the absorbing power of the cell¹ (Ursprung and Blum, 1916; Thoday, 1918).

Accepting the differential permeability of the upper and lower membranes of the sporangiophore of *Pilobolus* it may be compared with an artificial cell *A* (fig. 1), closed below by a perfectly semi-permeable membrane *B* and above by a membrane *C* which is slightly leaky, *i.e.* allows some of the osmotic material to diffuse out slowly. If such a cell is placed with its lower end in water, fluid will pass in,

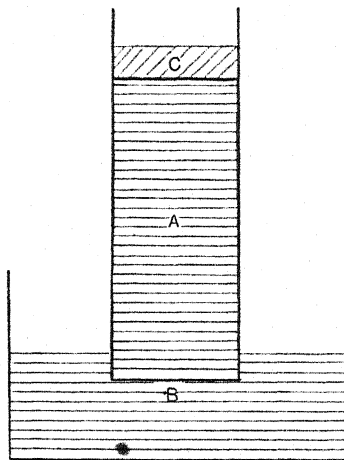


Fig. 1

and after a time the pressure exerted will equal the osmotic pressure of the contained solution. Water will then cease to enter, and the absorbing power of the cell will be zero. If now a drop of water be present above the upper membrane (*C*) osmotic substances will diffuse through the membrane and we shall have a weak solution above the cell. This solution will now have a higher absorbing power than the cell and so will draw water out of it. This loss will be immediately balanced by absorption through the lower membrane. The volume of the fluid at *C* will thus increase, *the force with which water is drawn into it depending solely on the difference of the osmotic*

¹ The absorbing power of a cell can be determined by finding the osmotic pressure of a solution which just does not alter the volume of the cell. This is the method used by Ursprung and Blum in their numerous measurements of the absorbing power of cells.

pressure of the fluids at *C* and at *B*, though of course the resistance of the two membranes will retard the rate of entry of the water. The osmotic pressure of the solution in *A* plays no part in the process, for *A* merely acts as a carrier of water and a reservoir of solute which can diffuse into the fluid above *C*.

In *Pilobolus* the excreted fluid is a weak solution containing no organic matter and with a concentration of mineral substances only one quarter that of the cell contents, so that unless the tension of the membrane comes into play the water from these drops will be drawn back into the cell—a difficulty by the way which Lepeschkin never meets. When the upper membrane of the sporangiophore becomes permeable some of the osmotic substances present in the cell sap will diffuse out into the water imbibed in the cell-wall. This weak solution would then draw water through the turgid cell in the manner just described for the model, and so the drops would be formed.

That drops of fluid might arise on the turgid sporangiophore of *Pilobolus* in this way seems clear, but the view that such a mechanism plays any important part in the actual process of exudation seems, on the other hand, very doubtful. The high temperature coefficient for exudation observed by Lepeschkin is rather against this view, though the effect might be due to the increased permeability of the protoplasm to water (see Delf, 1918). More definitely against the view is the fact that at a high temperature and under the action of alcohol the exudation may go on up to a point at which the turgor of the cells is markedly reduced, and it may even go on when the cell is partly plasmolysed by a 0.5 per cent. solution of NaCl. One would expect the exudation of fluid to stop before the turgor had been markedly reduced. The salt solution would certainly bring the absorbing power of the cell beyond the osmotic force of the drops (which is less than 0.35 per cent. NaCl), so that the continuance of the exudation, though at a diminished rate, is definitely against such a simple explanation of the mechanism. The process is probably a more complex one, depending on special energy relations of the living cell.

The hydathodes of *Phaseolus* and the secreting systems acting in root-pressure are essentially similar, but differ from *Pilobolus* in that the secreting cell or cells are separated from their water supply by a chain of intermediate cells. Now Lepeschkin claims that in order that water may pass to the apical secreting hairs of the *Phaseolus* hydathodes an osmotic gradient must exist with the apical secreting cells exhibiting the highest osmotic pressure; and Priestley appears

to assume such a gradient between the root-hair and the wood-elements. It has been pointed out in this *Journal* by Thoday¹ (1918), in an admirably clear and useful paper, that such a gradient is in no way necessary; all that is required in the chain of cells is a *gradient of absorbing power* with the highest absorbing power in the secreting cell; this might easily be consistent with a reversed *osmotic* gradient. Furthermore it may be pointed out that if the water supply is ample and there are no intermediate losses of water such a gradient will necessarily arise whatever the distribution of osmotic pressures.

If in fig. 2 we imagine that the root-hair, *A*, exposed to water has the highest osmotic pressure and the cells *B* to *E*, and the xylem vessel *F*, progressively lower pressures, water will still pass in from

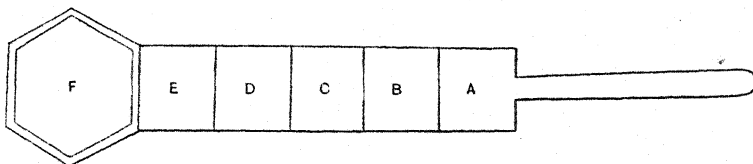


Fig. 2

A to *F*. For as *A* takes up water its absorbing power will tend to fall below that of *B*, when *B* will immediately take water from it, the absorbing power of *B* will then tend to fall below the osmotic pressure of *C*, and *C* will immediately take water from *B*, and so on; thus a gradient of absorption will arise. As the cells get turgid and cease to have any absorbing power, *F* will be able to draw water from outside *A*, *however low its osmotic pressure*, and the *force* with which water is drawn in will depend solely on the difference between the osmotic pressure of the contents of *F* and the osmotic pressure of the solution external to *A*. The osmotic pressure of the other cells can be neglected, though the resistance to water passage resulting from the interposition of these cells will reduce the rate at which water will pass from *A* to *F*.

The existence of an osmotic solution in the vessel *F* could be explained, as in *Pilobolus*, as a result of diffusion from *F* through the partially permeable membrane between the two. The accumulation of fluid under pressure in *F* would depend, as already stated, solely on the osmotic pressure of the solution in the vessel, and would

¹ Höfler (1920) has recently dealt with the water relations of the cell in a manner very similar to that of Thoday but somewhat more fully; he makes however no reference to Thoday's paper.

in no way depend upon the osmotic pressure in the adjacent living cells *E*, although such a dependence is usually assumed in theories of osmotic pressure. The objection to the assumption of such dependence may be put in another way—if the osmotic pressure of *E* is acting freely it would draw water back from *F*, if the pressure in *E* is opposed by the tension of the cell-wall it cannot exert pressure on the contents of *F*.

That the exudation of fluid from the open vessels of the wood could arise in this way seems clear, but just as in the case of *Pilobolus* it is very doubtful whether such a mechanism plays any part in the "bleeding" of cut stems. As with this mechanism the driving force depends on the concentration of the solution in the vessels we should expect some relation between the concentration and the pressure under which the fluid is excreted, but in the vine high pressures may be accompanied by a low concentration. In some cases also the fluid has almost the composition of "spring water" (Pfeffer, 1900, p. 262) which would mean a solution of very low osmotic force¹. The marked effect of deprivation of oxygen and possibly of temperature suggests also, as in the case of *Pilobolus*, that root-pressure cannot be explained by means of simple osmotic relations but that "vital" relations of the cell involving energy transference are really involved.

The value of Pfeffer's scheme in explaining exudation and root-pressure seems to have been overlooked of late years. It has the great advantage that, although it requires a supply of energy and thus calls upon the "vital activities" of the cell it will explain the exudation of pure water from the cell. In this scheme the osmotic substance is supposed to exist in the cell at two different concentrations. It may be represented by the model in fig. 3. The curved glass tube is closed by two completely semi-permeable membranes *A* and *B*. The arm above *A* is filled with a strong sugar solution (say $M/1$), the arm above *B* with a weak sugar solution (say $M/10$), the intervening space being filled with water; the two arms are supposed to be plunged in water. On the entry of water through *A* and *B* pressure will develop in the tube and when the pressure reaches

¹ Priestley (1918, p. 199) has suggested that in "bleeding" the soluble substances which pass into the vessel below are absorbed again during their passage up the stem. The value of any theory is, however, seriously weakened when it requires the support of a subsidiary hypothesis. Furthermore if we are to fall back on the unknown processes of "physiological absorption" the theory ceases to be a physical explanation of the process. The question also arises why should the cells below give out substances and the cells above reverse the process?

that of the osmotic pressure of the solution above *B* absorption through *B* will stop, since, owing to the pressure, the solution on the one side of the membrane *B* will be in equilibrium with the water on the other side. At *A* however there will be no equilibrium at the two faces of the membrane owing to the higher concentration of the solution and water will continue to enter. As a result the pressure in the tube will go on increasing and there will no longer be equilibrium at *B*, but water will be forced out and the solution in that arm will become more concentrated. If *B* were not in water but exposed to the air water would appear at its surface. A transference of water through the tube has occurred and so work has

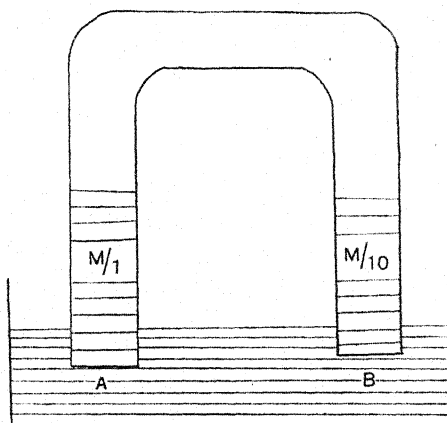


Fig. 3

been done, but there is no contravention of the second law of thermodynamics, for the process will not continue indefinitely as the two solutions will gradually mix; the work has been done at the expense of the energy of diffusion. If the process is to continue in the living cell work will have to be done to keep the two solutions at different concentration in different parts of the cell¹. An advantage of a theory of this kind is that exudation under high pressure could be obtained, for the exudation pressure would be the difference between the osmotic pressure of the two solutions. If the membrane in contact with the solution of lower concentration is slightly permeable the

¹ Rowell (1918) following Pfeffer has a somewhat similar model but it would seem to be unworkable since the two solutions of different concentration are separated by semi-permeable membranes.

substance could diffuse out and a solution under pressure would be forced into any vessel attached at *B*.

Hitherto normal osmosis has been considered but the question naturally arises as to whether these processes of exudation can be explained by the special properties of the membrane. It is known that some membranes will bring about a "negative osmosis" in which fluid will pass from a strong solution into a weak one just as it does in the case of *Pilobolus*. There is, for example, the work of Bartell (1914) who found that porcelain membranes with pores of large size showed an increase of pressure on the side of the weak solution, while similar membranes with finer pores showed a normal pressure. Bartell and Madison (1920) have examined the effect of gold-beaters' skin used as an osmotic membrane. They found that with various solutions the normal osmotic tendency might be increased, decreased or reversed. The results can be explained by the electrical relations of the membrane; a difference of potential between the two faces of the membrane is developed if electrolytes are used and this electro-endosmosis may aid or retard the normal process of osmosis; we thus have an additional force superimposed on the ordinary osmotic relations as indicated earlier. For the production of negative osmosis energy must be available so that, as pointed out by Freundlich (1916), the phenomenon can occur if the membrane is permeable to the electrolyte as a whole, or if it is permeable to one ion only of the electrolyte, and the electrolytes on the two sides of the membrane are different and react with one another. The gold-beaters' skin membranes are leaky membranes permeable to the electrolytes as a whole so they fulfil the first condition.

The membranes of the living cell are generally more or less permeable and they are bathed with electrolytes. Differences of electrical potential can be detected in living tissue, so that the negative osmosis to be observed, through such a membrane as gold-beaters' skin, may be similar in origin to the negative osmosis which is characteristic of the exudation exhibited by the sporangiophore of *Pilobolus* and by hydathodes of the type of those on the leaf of *Phaseolus*. It is, however, doubtful if exudation under the high pressures sometimes associated with root-pressure can be explained in this way.

An attempt has been made to show that the claim of Lepeschkin that the osmotic pressure of the stronger cell contents is responsible for the exudation from the cell of a weaker solution cannot be substantiated. A number of mechanisms can, however, be suggested

by means of which this exudation could be brought about, though how far such mechanisms are actually at work in the living cell it is at present impossible to say. Much more knowledge of cell dynamics is required before we can deal satisfactorily with such difficult problems as exudation and root-pressure. Quantitative data are particularly necessary for example as to the force with which fluid can be exuded from the cell as the result of the action of electro-endosmosis or of the difference of concentration of solutions in different parts of the cell.

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THE REVERSAL OF GEOTROPIC RESPONSE IN THE STEM

I. THE EFFECTS OF VARIOUS PERCENTAGES OF CARBON DIOXIDE

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(With Plate I.)

THIS work was undertaken with a view to obtaining further experimental evidence, bearing upon the Hydrion Differentiation Theory of Geotropism which was recently brought forward by Professor Small (*New Phytologist*, **19**, p. 49, 1920). In particular, that aspect of the theory which relates to the carbon dioxide of respiration as a differentiating factor in the growth curvatures of geotropism was in mind (*cf.* Small and Rea, *ibid.* **19**, p. 208, 1920).

According to this theory it is the hydrion concentration of the continuous phase of the protoplasm in the cells of the region of gravity perception, which determines the direction of the curvature resulting from response to the stimulus.

Under normal conditions the stem, according to this theory, is a relatively alkaline structure, because the carbon dioxide of respiration does not accumulate (most of it being used up by the plant during photosynthesis), and one result of the relative alkalinity of the continuous phase of the protoplasm in the perceptive cells is that a horizontally placed stem under normal conditions turns upwards in response to the stimulus of gravity.

If the conditions are changed so that the horizontally placed plant is growing in an atmosphere made acid by the addition of an excess of carbon dioxide, then, as a result of the accumulation of the carbon dioxide within the stem, the stem may be rendered less alkaline and the hydrion concentration of the continuous phase of the protoplasm in the perceptive cells may be raised to such a degree that the direction of the geotropic curvature will be affected. The experiments described below were carried out with the object of

discovering whether the curvature was affected under such conditions. The method of procedure in the experiments was to place the plants horizontally in an atmosphere to which known percentages of carbon dioxide had been added, and to determine the effect of such abnormal conditions on the geotropic curvature of the stem.

In the course of the work it became apparent that, with added amounts of carbon dioxide above a certain percentage, there was a very decided reversal of geotropic response, the stem structures being actually positively geotropic.

It was also equally apparent that with added quantities of carbon dioxide lower than this "certain percentage," the response to the stimulus of gravity resulted in quite a normal negatively geotropic curvature.

It therefore became necessary to determine, as closely as possible, the minimum percentage of carbon dioxide which had to be added so that there would be reversal of the normal curvature.

The different stages in the development of the work are described below.

A number of preliminary experiments were carried out in October 1920. In the first one conducted, two healthy seedlings of *Clarkia elegans*, growing in garden-soil in a box, were placed horizontally in a glass jar into which carbon dioxide was being passed at the top and from which there was an outlet at the base.

After the lapse of one day no change was noted in the seedlings, *i.e.* the stems were still quite horizontal. At the end of two days both seedlings showed a slight downward curvature of the stem and on the third day there was a very strong downward curvature.

On the fourth day the seedlings had an upward turn. This last change was probably due to the fact that, after the end of the second day, the supply of carbon dioxide was cut off from the jar, and since the jar was not darkened in any way the seedlings were able to carry on photosynthesis, thus using up the carbon dioxide from the atmosphere in the jar (which in addition was not air-tight), so that on the fourth day the atmosphere in the jar was almost back to normal. The stems of the seedlings would then become relatively alkaline again and so show the normal negatively geotropic curvature. This case of recovery may be compared with other instances to which reference will be made below.

Further preliminary experiments were carried out with seedlings of *Clarkia elegans*, *Antirrhinum* and *Helianthus*; from these experiments it became quite clear that reversal of normal curvature

resulted when carbon dioxide was present in certain quantities in the atmosphere.

It was some time before the best method of procedure was arrived at, and since these preliminary experiments were conducted on less accurate lines, they will not be referred to further in spite of the fact that the results were in accordance with the theory.

It was found, in the course of preliminary experiments, that the seedlings which gave the best results were those of *Helianthus annuus*, because they respond so readily to the stimulus of gravity under normal conditions, and because they are even more ready to show an abnormal curvature under abnormal conditions.

The following method was the one adopted in all the experiments tabulated below: A box containing sand in which were growing a number of erect, healthy seedlings of *Helianthus* was fixed in a large bell-jar so that the hypocotyls were in a horizontal position. (The bell-jars used in the experiments varied in volume from 4000 c.cs. up to 9000 c.cs.) A ground glass plate was sealed to the bottom of the jar with paraffin wax and carbon dioxide was introduced by displacement of a known volume of water, giving a percentage which was known approximately. The jar containing the seedlings was placed in a dark cupboard, in order to avoid heliotropic effects, and was examined at regular intervals. One of the properties of carbon dioxide is that it diffuses rapidly and, this being so, it may be taken that the gas in the jar was of uniform concentration throughout. Samples of gas from the top and bottom of a jar were analysed and proved to contain the same percentage of carbon dioxide. Further, as soon as a curvature in any direction was noticed, a sample of the gas in the jar was analysed. The apparatus used in the analysis of the gas in each case was a modification of the Hempel Apparatus for Gas Analysis.

The experiments carried out are tabulated below, and in each case the method adopted was exactly similar to that described above.

Date of experiment	Number of seedlings	Number of seedlings showing curvature	Volume of jar in c.c.	% CO ₂ (by analysis)	Temperature ° C.
1. 1st Dec. ...	5	5 down	4000	33	14
2. 11th Feb. ...	7	7 "	7000	32	12
3. 11th Feb. ...	9	9 "	7000	29.7	12
4. 17th Jan. ...	3	3 "	4000	26	13
5. 19th Nov. ...	3	3 "	4000	25	14
6. 11th Feb. ...	4	4 "	7000	22	12
7. 25th Nov. ...	3	3 "	4000	20.9	14
8. 30th Jan. ...	2	2 "	9000	20	13
9. 19th Jan. ...	5	5 "	4000	19.6	13
10. 1st March ...	5	5 "	7000	18	12
11. 27th Jan. ...	2	2 "	9000	16.8	14
12. 22nd Nov. ...	7	6 "	4000	16	14
13. 21st Jan. ...	5	5 "	4000	14.5	13
14. 7th Feb. ...	3	3 "	7000	14	11
15. 7th Feb. ...	10	9 "	7000	13.3	11
16. 20th Jan. ...	4	4 "	5500	12.6	14
17. 21st Feb. ...	2	2 "	7000	10.5	13
18. 18th April ...	7	7 "	7000	10.4	14
19. 1st Feb. ...	2	2 "	4000	10.3	13
20. 14th March ...	6	6 up	7000	9	14
21. 1st Feb. ...	2	2 down	4000	9	14
22. 14th March ...	7	7 up	7000	8.2	14
23. 25th Feb. ...	4	4 down	7000	8	14
24. 10th March ...	7	1 down 6 up	7000	7.9	13
25. 11th March ...	7	7 up	6250	7.76	15.5
26. 16th March ...	9	9 "	7000	7.6	14
27. 21st Feb. ...	2	2 down	7000	7.4	14
28. 17th Feb. ...	9	9 up	7000	7.2	14
29. 15th March ...	7	7 "	7000	7.1	14
30. 25th Feb. ...	5	5 "	6250	7	14
31. 25th Feb. ...	8	8 "	9000	6	14
32. 14th Feb. ...	2	2 "	7000	5.2	13
33. 15th March ...	9	9 "	7000	5.2	14

NOTES ON EXPERIMENTS

Exp. 1. The box containing the seedlings was removed from the jar on Dec. 2nd and was left horizontally in fresh air and light with the hypocotyls of the seedlings still showing the strong downward curvature. On Dec. 3rd all the seedlings showed a normal negatively geotropic curvature. This complete recovery was brought about because photosynthesis was carried on when the seedlings were in ordinary air and light, and thus the accumulated carbon dioxide was used up, the hypocotyls became again relatively alkaline and normal upward curvature resulted.

Exp. 2. All the seedlings showed normal upward curvature when left in ordinary air and light—as in the first experiment tabulated.

Exp. 3. All seedlings showed normal curvature when taken out of jar and left in air and light.

Exp. 5. Seedlings showed normal geotropic curvature after having been left in air and light for a day.

Exp. 12. These seedlings were photographed in the jar, when showing reversed curvature (see Plate I, fig. 1). The carbon dioxide was removed from the jar on the afternoon of Nov. 22nd and the seedlings left in air and light. On the following morning all the seedlings showed a normal upward curvature. They were again photographed (see Plate I, fig. 2). The hypocotyl of the 7th seedling was too short to show curvature.

Exp. 15. Nine of these seedlings showed a very definite curvature downwards at first, but after some hours two of the seedlings showed a negatively geotropic curve. The seedlings were photographed while still in the jar. An examination of Plate I, fig. 3 will show the two seedlings referred to above, and it is clear from the photograph that the first curvature was in a downward direction. The box of seedlings was taken out of the jar and after twelve hours in air and light the hypocotyls of all the seedlings showed a normal negatively geotropic curvature (see Plate I, fig. 4).

Exp. 20. These seedlings had been brought on in bright light, hence because of active photosynthesis the carbon dioxide of respiration had not accumulated to any extent before the seedlings were put into the jar. This fact would probably account for the normal curvature, because a higher percentage of carbon dioxide would be necessary to decrease alkalinity sufficiently to produce reversed curvature (see below).

Exp. 22. After four hours in the atmosphere containing 8.2 per cent. carbon dioxide, two of these seedlings showed a slight upward curvature and the remaining five were still quite horizontal. On the following morning all the seedlings had a normal upward curvature. Again in this case the seedlings had been brought on in bright light.

Exp. 23. The downward curvature was slight in these seedlings, and was not apparent until several hours had elapsed.

Exp. 24. In this experiment, response to the stimulus was much slower than the abnormal response in the higher percentages. When the six seedlings were showing normal geotropic curvature, an additional amount of carbon dioxide was passed into the jar, and the jar was replaced in darkness. After two days the hypocotyls of the seedlings (which with 7.9 per cent. carbon dioxide in the atmosphere had shown a normal curvature) had turned downwards, because the addition of the extra carbon dioxide to the jar had rendered the atmosphere sufficiently acid to raise the hydrion concentration in the perceptive cells to an extent sufficient to cause reversal of the normal curvature.

Exp. 27. These seedlings had been brought on in fairly dull light, so that there would be little photosynthesis, and therefore CO_2 would have accumulated to a certain extent. This would probably account for the reversal with the small percentage (see below).

From the results tabulated above it is quite clear that reversal of geotropic curvature actually does occur in the hypocotyls of seedlings of *Helianthus annuus*, when the seedlings are fixed horizontally in an atmosphere containing from about 9 per cent. to 30 per cent. of carbon dioxide.

With the apparatus employed in the analysis of the gas it was found that an error of 0.1 c.c. in the reading of the volume of the gas remaining after the CO_2 had been absorbed from the sample, resulted in an error of 1 per cent. either way in the percentage of carbon dioxide as analysed; when allowance is made for such an error (*i.e.* 1 per cent. in either direction) it seems reasonable to conclude from the results tabulated that the percentage of carbon dioxide necessary to produce reversal of the normal geotropic curvature in the hypocotyls of *Helianthus annuus* is approximately 7 to 10 per cent.

The minimum percentage of carbon dioxide necessary to produce such abnormal response would no doubt be slightly different even for different plants of the same species, *e.g.* in cases where the previous history of the plants used in the experiments had differed.

The factor in the previous history which would have the greatest effect would be light. If the plants were brought on in bright light then the CO_2 of respiration would be used up in photosynthesis, and, therefore, would not have accumulated to any extent in the plant. On the other hand, photosynthesis would be much less active in plants brought on in dull light. Such plants would have accumu-

lated to a certain extent the carbon dioxide of respiration, and in such a case a smaller percentage of carbon dioxide in the atmosphere would alter the alkalinity of the stem sufficiently to cause a reversal of the normal geotropic curvature. Reference is made in the "Notes on Experiments" to cases in which such previous history may have affected the direction of curvature.

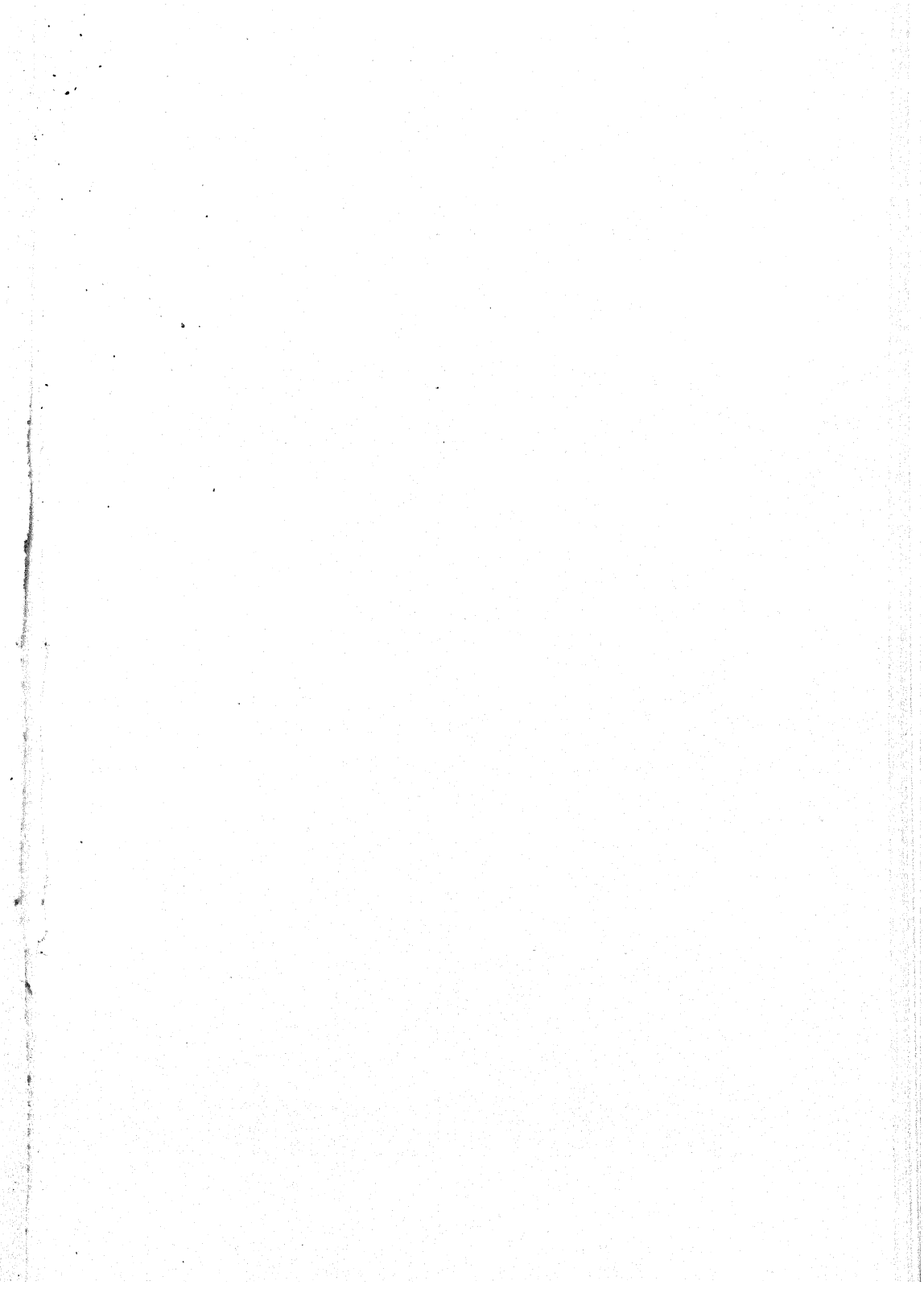
In order to prove that the downward curvature of the *Helianthus* seedlings in the atmosphere containing carbon dioxide above a certain percentage really represents a reversal of geotropic curvature, experiments were carried out with a klinostat.

A box containing seven *Helianthus* seedlings was attached horizontally to a revolving klinostat. The klinostat used in these experiments had a long horizontal rod as the revolving axis, and this axis was fitted through a hole in the cork of a jar which was supported horizontally and was similar to those used in all the above experiments. The box containing the seedlings was clamped horizontally at the end of the revolving axis of the klinostat and the jar was made water-tight by means of a glass plate and paraffin wax as before. Carbon dioxide was introduced by displacement of water as before to the extent of approximately 30 per cent.

In the previous experiments in which the percentage of carbon dioxide was as high as 30 per cent. reversal of geotropic curvature was evident after a very short time ($\frac{1}{2}$ to $\frac{3}{4}$ hour), but when the seedlings were being revolved on a klinostat in an atmosphere containing 30 per cent. CO_2 there was no curvature. In this first experiment with the klinostat the seedlings were watched carefully for 6 hours, and at the end of that time they were still absolutely horizontal.

This experiment was repeated on several successive days with different sets of seedlings, with the same result, *i.e.* no curvature on the klinostat. If the downward curvature of the seedlings in the atmosphere containing above 10 per cent. of CO_2 were *not* a geotropic phenomenon, there would be curvature even when the seedlings were being revolved on the klinostat. It has been shown by these experiments with the klinostat that no curvature takes place under these circumstances, and therefore the downward curvature in all the experiments in which it occurred quite clearly represented a reversal of the normal geotropic curvature.

In conclusion, I should like to express my gratitude to Professor Small, under whose supervision this work has been carried out and whose suggestions and criticisms have been invaluable.



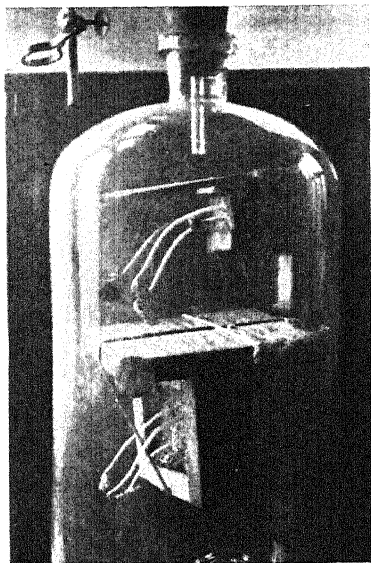


Fig. 1

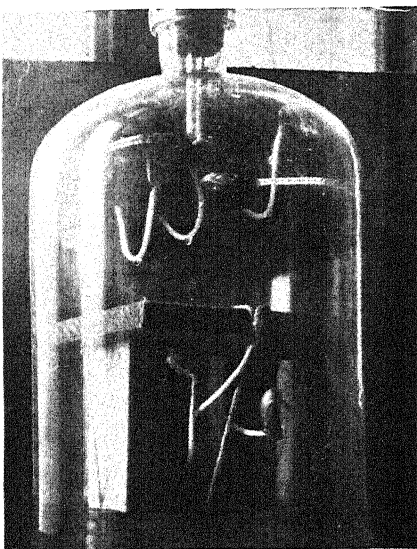


Fig. 2

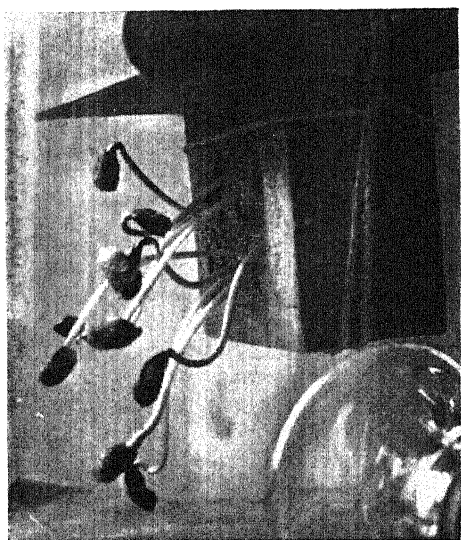


Fig. 3

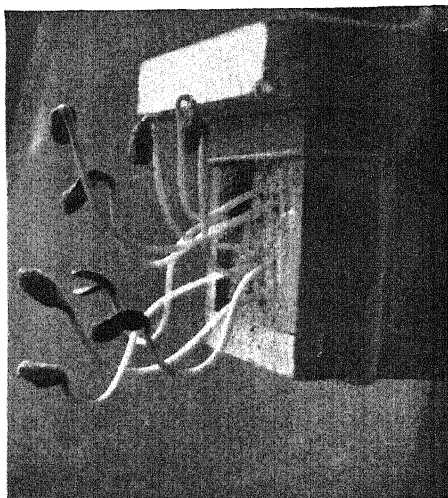


Fig. 4

LYNN—EFFECT OF CARBON DIOXIDE IN REVERSAL OF
GEOTROPIC RESPONSE

EXPLANATION OF PLATE I

- Fig. 1. Seven sunflower seedlings, six of which show reversed geotropic curvature in 16 per cent. carbon dioxide (Exp. 12).
- Fig. 2. The same about 24 hours after the excess of carbon dioxide had been removed, showing recovered normal geotropic curvature.
- Fig. 3. A similar set of sunflower seedlings most of which show reversed geotropic curvature in 13.3 per cent. carbon dioxide (Exp. 15). One of the seedlings shows normal curvature, two show recovered normal response as well as a downward curvature.
- Fig. 4. The same about 12 hours after they had been removed and placed in fresh air and light, showing recovered normal geotropic curvature.

REVIEWS

GENETICS AND EVOLUTION

Hagedoorn, A. L. and Hagedoorn, A. C. *The Relative Value of the Processes Causing Evolution*. Pp. 294. Martinus Nijhoff. The Hague, 1921. Price 9 guilders.

The authors of this very interesting book, on the basis of a long personal experience of genetic research, a wide acquaintance with the literature, and—not least important for their task—an evidently extensive knowledge of the world, of the different nations, races and classes of man, and of the breeds of domestic animals and plants, discuss afresh the still unsettled question of the factors of organic evolution. The following review does not profess to be critical. It aims simply to give the ordinary botanist some idea of what the book contains.

The authors call attention to the wide divergences of opinion still existing as to the factors of evolution, in spite of the enormous development of genetic research during the last 20 years, and to the reluctance of geneticists to make any attempt to correlate the facts so far obtained. This reluctance is not quite universal. The authors pay tribute to Bateson's subordination of the different specialised genetic investigations to the main problems of evolution¹. "The first author," they say, "after Darwin who approached genetics in this spirit was Bateson." And Gates, in the pages of this *Journal*², and elsewhere, has recently made similar attempts. It may be urged of course that we still do not know enough of the facts to theorise safely about evolution and that we must await the results of further research before attempting to do so. It is clear in any case that general theorising about evolution must still be provisional. It can only be a question of reviewing, from time to time, the knowledge accumulated, and of endeavouring to see where we stand. This the ordinary biologist is unable to do for himself owing to the extremely specialised work of the modern geneticist, and the complicated and unfamiliar language in which it is expressed.

Meanwhile it is evident that the subject is still liable to the over-emphasis of single factors on the part of individual theorists. "There is one point common to all the theories of evolution, excepting Darwin's, and that is that each theorist has always over-emphasised one point, one single link in the chain of processes which goes to the making of species, and has brought out this point as 'the' cause of evolution. Just as Lamarck gave all his attention to adaptation [*i.e.* direct adjustment to environment] and led himself to believe that adaptation is the cause of numerous processes with which we now believe it to be

¹ *The Problems of Genetics*, Yale University Press, 1913; Presidential Address to the British Association, London, 1914.

² "Mutations and Evolution," *New Phytologist*, 19, 1920. (Reprint, No. 12, 1921.)

only remotely concerned, so has Weismann over-emphasised the power of selection to the exclusion of everything else. And de Vries, who believed he had witnessed a striking instance of mutation, spontaneous origin of species, has come to believe mutation to be the sole important cause of evolution." But the modern geneticist, for the most part, takes little interest in the problems of evolution, perhaps by way of reaction from the excessive speculation and fine-drawn theorising of the post-Darwinian period at the end of last century. "No one, since Darwin," our authors consider, "has set forth a comprehensive theory of evolution worth the name."

"Every theory of evolution must account for variation, must give a plausible explanation of the causes of that variation which may be instrumental in species formation, and in the second place it must account for specific stability. . . . Lamarck thought that the stability of a species is obtained. . . . as soon as the species has come into a new state of equilibrium with its surroundings. . . . Weismann thought of the final stability and purity of a species as the result of a long continued natural selection. De Vries holds that the unknown causes for the abrupt variation which produces a new species imply a new stability."

In regard to heredity the authors rightly lay stress on the great advance made by Roux when he distinguished determination factors from realisation factors. We now believe, as has been said, that "every character of an organism is both inherited and acquired." "Genetics," according to the Hagedoorns, "is essentially a branch of biomechanics, concerning itself with a study of those factors in the development of an organism which are inherited." They believe that "numerous things are transmitted from parent to offspring, each of which, by its presence in the cells, tends to influence one or more definite steps or processes in development, whenever these steps are taken or these processes undergone. . . . no latency or semi-latency need be ascribed to these inherited things (*genes*), which in certain individuals are not factors in development and which nevertheless are transmitted by them to some of their children¹." All inherited "factors" are genes but all the genes present in the germ are not necessarily factors in its development. The presence or absence of a certain gene may determine a definite difference in the final qualities, but it is inadmissible to speak of such a gene as the "determiner" of that quality, for all the other genes contributing to the developmental process which results in the character in question could each, and all in their turn, be called its determiner. The characters of an organism are not so many separate things, they are all the result of a great many factors, some inherited (*genes*), some furnished by the environment. We have every reason to believe now that every gene is present in the zygote in the same state, that every zygote is a fresh beginning, that in so far as an individual's character can be said to be determined in its germ, it is given in the combination of genes present, not in peculiar states of them.

"A vitalistic view of the nature of genes certainly fits the facts, but whereas

¹ Thus for instance there is a gene in rats, which when present in coloured animals makes otherwise black animals "agouti," but in albino rats the same gene, though it has no influence upon colour, is nevertheless transmitted in precisely the same way as in families of animals in whose development it plays an active part.

it is a theory that will work, it is not a theory one can work with." It has been shown, conclusively the authors think, that heritable variability is synonymous with genotypic impurity. In those cases where we are sure that the origin of the group ensures purity of the genotype selection has been shown to be ineffectual (Johannsen's law). Thus we do not need to make provision, in our theory of the nature of genes, for qualitative variability in the genes themselves. And this point, in their view, is the only justification for the supposition that the genes are protoplasmic. "Protoplasm is clearly an emulsion, and it must be ultimately made up of a number of non-living substances, the combination of which makes it living." The attitude of the vitalist who reasons that every constituent of protoplasm which is an integral part of it and which shows one or more of the properties of protoplasm is itself protoplasmic may be compared to the attitude "of a philosophically minded eater of plum-pudding, who should argue that the round sweet things he could dissect out of his helping, and which looked like raisins, could not *be* raisins; since he found them in his plum-pudding and forming an integral part of it they must *consist* of plum-pudding."

Quantitative propagation combined with qualitative stability is not exclusively a property of protoplasmic bodies multiplying by bipartition. Autocatalytic chemical substances fulfil both requirements, since they propagate themselves, *i.e.* suitable materials are changed into a new substance under the influence of that substance. Also they remain qualitatively unchanged¹. The theory that genes are of this nature is believed by the authors to be compatible with all the facts known about the action of genes.

All the circumstantial evidence certainly points to the conclusion that the chromosomes are ultimately bound up with the process which leads to a distribution of the genes over cell generations, and there is no incompatibility between the view that the genes are of a relatively simple chemical nature, and that they are in some way localised in or on the chromosomes.

We may perhaps suppose that a complete set of genes is kept intact inside the nucleus, and we must probably take the view that there is no real difference between the nuclei of "germ" cells and those of "somatic" cells. The facts of regeneration of the whole plant from single somatic cells are fairly decisive for this view. But the cytoplasm of individual somatic cells of different tissues certainly has strikingly different chemical, physiological and morphological properties, and this may be due to the quantitative preponderance of one or two genes (autocatalysts) in extremely differentiated cells.

We cannot directly compare a multicellular with a unicellular organism. The cells of the former that are in immediate contact with the environment have, as a rule, no "future," *i.e.* they have no germ cells among their descendants. If we conceive of a gene as simply a chemical substance, the taking up of a new gene by a unicellular organism may not be an impossible or a rare process. But it is significant that, according to our authors, *no authentic case of a positive mutation* in the higher plants or animals is on record. And we must beware of accepting instances of the effect of selection in apparently pure clones of unicellular organisms as invalidating Johannsen's law.

¹ See A. I. Hagedoorn, *Vorträge und Aufsätze über Entwicklungsmechanik Roux*. Leipzig, 1911.

The occurrence of domestic species with new dominant characters is no argument for the occurrence of positive mutations, for such species may be the result of crossing; and even if the species crossed had not the character in question there is ample evidence that genes may be transmitted from parent to offspring through many generations without contributing to the qualities of the individuals. The hybrid between two very similar, almost identical, subspecies may have a very striking new dominant character.

The authors show that either continuous or discontinuous "variation" (in the wide sense of "difference") may be caused by a change in the genotype or by differences of environmental factors or by both acting together. So that it is impossible to suppose on the one hand, with Darwin, that only continuous variation is important in evolution, or on the other, with de Vries, that only discontinuous variation has evolutionary significance.

For the production of a character the co-operation of many genes is necessary, and the lack of any one will lead to a failure to develop a character. We cannot speak of that one which happens to be absent as the determinant of the character, any more than we can speak of one link of a chain from which a weight is suspended as holding up the weight. We can only study those genes which are in some cases absent. So long as a gene is invariably present we cannot investigate its genetic behaviour. And many genes, viz. those which are essential for the working mechanism of the organism, must be universally present. But it is possible to acquire the faculty of distinguishing differences caused by difference of genotype from those caused by environmental conditions by constantly observing, for instance, the differences between pure clones, e.g. nursery trees. These differences are often *made up* of apparently trivial points which might be considered as part of the fluctuating variability of the plant and attributed to differences of conditions. But the members of each clone are in fact often remarkably constant—their variability is very small. And selection between these members has no effect on the offspring. Selection is of no effect in a population without genovariability, and therefore in such a population it cannot accumulate a difference and lead to a modification of the race.

The authors take the view that dominance implies the presence of a gene which is absent in the recessive type. They counter the argument that a new dominant character must, on this view, be due to the appearance of a new gene, by referring to their contention that no gene can influence development unless there is present a given combination of other genes and non-genetic developmental factors. This is illustrated by the production of red pigment in *Primula sinensis* and a dominant yellow in mice. The case of the well-known Emily Henderson sweet peas is another instance. So that if a dominant novelty appears we cannot decide offhand that a new gene has appeared; it may be that two or more genes, none of which could produce the new dominant character unless all were present, have met and have thus produced it. They insist that we are forced in any case to look upon the production of every character as resulting from the co-operation of numerous genes, and that to consider such cases as special "complications" is a wholly mistaken view which had its origin in the fact that the first cases investigated depended on the simple presence or absence of one gene which when present completed the chain necessary for the development of a striking character.

With regard to the production of a red-fruited form in *Oenothera*, recorded by Gates, which appears to be a dominant novelty originating independently of crossing, the authors argue that since these *Oenotheras* show a remarkable suspension of combination and segregation in hybrids, characters being separately transmitted through the pollen and through the ovules, it is quite possible to suppose that in a stock originating by crossing in which this suspension had been maintained for many generations, a failure of the suspension mechanism might give rise, by the combination of genes hitherto kept separate, to a dominant novelty. In *Drosophila* also, the fly which has formed the material of so much recent genetic work, there are obviously so many irregularities in the ordinary segregation processes that it would not be surprising if Morgan's dominant novelties turned out to be produced in a somewhat similar way to that suggested for the red-fruited *Oenothera*.

Only if we are absolutely certain that the individuals of a given family are homozygous can we call the sudden production of unexpected novelties mutations in the strict de Vriesian sense. It must be noted that in America especially the term mutation is sometimes used in a much looser sense, *i.e.* as the production of *any* "novelty." The authors incline to the view that while no case of the acquisition of a new gene (positive mutation) has really been proved to occur, the loss of a gene (negative mutation) has occurred in real "pure lines" (Johannsen, Nilsson Ehle) though in animals it is well-nigh impossible to distinguish between this phenomenon and the production of a recessive novelty through rearrangement of genes.

A very important part of the authors' general theory of evolution is based on their view of the relation of heritable variability to groups of individuals (clones, families, domestic breeds, or colonies, varieties or species in nature). They use the phrase "total potential variability" for the number of genes in respect of which such a group is not pure. The potential variability of a group of organisms increases if there are taken up into the group individuals which either possess a gene or genes not present in any member of the group, or which lack genes that are the common property of all the members of the group. Potential variability differs from variability because it concerns all genes, whether they have been factors in development or not. The authors believe that the total potential variability of any group is always tending to decrease (in the absence of crossing with other groups) because the offspring of any generation are normally produced, both in nature and under domestication, from a small fraction only of the individuals of that generation; and this must lead to the dropping out from the breeding stock of part of the original total potential variability of the group. This will nearly always happen, even where the new generation is derived from a random sampling of the old, because the chances are that some combinations will be omitted, and at a greater rate of course if it is the result of a definite selection from the old. But the reduction of potential variability does not depend upon selection, it is automatic, so long as some individuals are dropped out of the breeding stock.

In the case of a self fertilised plant the reduction of variability will proceed (averaging 50 per cent. in each generation) even if all the individuals produce offspring, because Mendelian segregation will result in the daughter being heterozygous for one half the number of genes for which the mother was impure. Only in the case of a quite freely crossing group of allogamous

organisms will the potential variability of the group remain undiminished (Jennings, Pearl), but such a condition assumes indefinite increase of the group which can never in reality occur for any long period. In a word heterozygotes will produce homozygotes, but not the reverse, and thus heterozygotes will *always* tend to decrease in successive generations of a group, unless crossing is universal throughout the group and all the offspring breed. This, according to the authors, and not selection is the real explanation of the purity of species, though selection may decide the ultimate genotype for which the group will become stable. In this connexion it is interesting to note that the authors are decidedly inclined to the view that in the normal case an "adaptive" character is developed owing to causes which have no reference to its "adaptive" nature, and that selection acts by enabling the organism to occupy a new habitat as a result of the possession of the character, not by developing the character in the first instance. Botanists are becoming increasingly familiar with instances which clearly point to this mode of origin of "adaptation to environment."

It is clear that on this theory isolation of a small colony from any group will speedily result in the purity of the genotype of the colony, which will differ from the genotype of the parent group, because the total potential variability of the small colony will be very much less and will differ qualitatively from the total potential variability of the parent group, and this will happen whatever the factors bringing about isolation (geographical, physiological, or the action of the breeder). If small colonies of plants belonging to one species establish themselves on so many islands, there are at once produced so many new "species" each of which rapidly becomes pure. Adaptation may have no part at all in such a process of species differentiation, because the characters in which the new species differ from one another and from the parent species may have no survival value in any of the habitats. On the other hand if the habitats differ and there is variability in the genotype corresponding with characters which have survival value in relation to the difference of habitat, there will be a selection which will play its part in determining the genotypes of the new species.

Thus we can understand why it is that geographically isolated but clearly allied species may or may not differ in "adaptive" characters. We can also understand how it is that different closely allied species come to exist in the same geographical area but in different habitats, different "ecological niches," between which the chances of crossing are at a minimum, *without* any obvious special adaptation to their respective habitats, and in other cases *with* such obvious adaptation. Where such habitats abut on one another one often finds "intermediates" of hybrid origin.

"Isolation of some sort is necessary; without isolation even selection cannot work against the levelling effect of the factors tending to reduce the potential variability." And any kind of isolation must tend to species formation, to the production of new groups, having their own "centres of stability" (Wagner), provided there is an unequal division of total potential variability in the isolated groups. In plants which are self-fertilised or apogamous as a rule, but where crossing is not absolutely excluded, numerous species may come to exist in the same area and the same ecological niche, for the changes in genotype brought about by crossing will be stereotyped by the subsequent isolation

of different families. In such cases when crossing occurs some of the species may disappear and others originate.

Species formation is also possible in allogamous organisms inhabiting a large area where barriers are absent but the rate of dispersal is very slow (e.g. snails in a lake), for there the conditions in every spot will approach those of complete isolation, owing to the sedentariness of the local populations, so that a great number of local forms, the despair of the systematist, may come to exist. Very close local study will certainly distinguish a number of these local forms, while the "lumper" will include the whole series in one species. Organisms with greater powers of dispersal are much more likely to be monotypic over a wide area (e.g. trout in the same lake).

These considerations lead on to the question as to whether, in the light of modern genetic research, we can obtain a logical and workable definition of the term *species*. The authors define it as a group of organisms so constituted and situated that they tend, under conditions which promise to be permanent, to reduce automatically their potential variability, and this is claimed as corresponding well with the conception of the taxonomist. The usual procedure of the taxonomist, the description of a "type specimen," is certainly founded on the common experience that an enormous majority of the plants or animals grouped under the name conform to the description. The prevailing opinion among systematists is that species are realities, real groups of organisms, of which the majority are true to type, and it is unlikely that this opinion is so devoid of foundation as some modern geneticists would have us believe. If the authors are correct in their main contentions we can see that it must correspond with reality; and that it is due to the fact that in spite of crossing there is always operative in greater or less degree an automatic reduction of the variability of each group of individuals which we call a species. A variety, on the other hand, differs sufficiently from the specific type (owing to chance combination of gametes both lacking a certain gene, or supplementing one another's genotype and thus producing a new character) to demand a name, but has no permanence—it does not commonly reproduce itself as such for many generations, but is reabsorbed into the species. A variety may become a species *if it is isolated*. Thus in autogamous plants there will be no varieties. For instance Jordan's "elementary species" of *Erophila vulgaris* (*Draba verna*) are real species.

According to Darwin there is no fundamental distinction between varieties and species—varieties were for him incipient species. This is only true, according to the authors, if the condition of isolation supervenes which it generally does not. Darwin clearly showed that there is no fundamental difference between the points which distinguish species in nature and those which separate breeds of domestic animals or strains of cultivated plants. In this last contention, according to the authors, he was perfectly right. But, they add, domestic breeds *are* species. They fulfil the conditions of the definition in every respect.

In their final chapter the authors consider the status of man, and the races and nations of mankind in relation to their general view of evolution and species formation, and they are forced to conclude that the factors which keep populations or groups within a population apart have the same effect as among other animals. The logical deduction would seem to be that not only do the different nations contain separate species of man, but that all groups within a

nation which will not or do not generally intermarry are also species. The existence and relative permanence of such groups rest upon many varied grounds, but very largely on the feeling of likeness between the members of each group and the feeling of difference between their group and other groups—a likeness and difference that may depend on race, religion, occupation, mode of life, possession of land, or similar factors. To set against these differentiating factors we have the widespread desire of the bulk of the members of a nation to be alike, to be one, at least in certain respects, and even the idea of "the brotherhood of man." Mass-immigration has tended to keep the population of the United States (except the negroes and the Japanese on the Pacific coast) "one species" in spite of the great diversity of its origin, because the immigrants are, on the whole, successfully absorbed. It is probably more truly one species than the population of any other great country.

The authors rightly insist that a study of these things is essential if we are to apply the results of genetic science to political machinery, and they deprecate the exclusive attention of the eugenists to single human characters (particularly defects) of no more than varietal value at most, even when hereditary, rather than to the natural group units of the human race, which have arisen and will continue to arise as the inevitable result of universal biological and psychological processes.

The authors have scarcely taken into consideration the direct effect of environment on organisms which many botanists, in particular, hold to be one of the most important factors of evolution, though they do refer to the possibility that mutations might be induced by varying the composition of water culture solutions. This view is much more prevalent among botanists today than it was 30 years ago, when the Darwinian influence, and especially Weismann's version of Darwinism, were at the height of their power, though many botanists have always held to it. If genes are really chemical substances, it is certainly possible to believe that the long continued influence of a given environment may favour in the protoplasmic complex the production of one or several genes (for instance by dissociation or combination or by dissociation and recombination) at the expense of others. A varying "set" of metabolism may be initiated by a given environment and inherited, if only through the cytoplasm of the egg-cell; and this means the increased production of certain substances and the decreased production of others. For instance the peculiar carbohydrate metabolism of typical succulent plants (Cactaceae) leading to the accumulation of pentosans and thus to the characteristic succulent phenotype might be a case in point. But it is true that we cannot exclude the possibility that the *origin* of this type of metabolism, which has been shown to exist in plants that are not succulent in the normal habitat, may depend on a segregation of genes without any initial relation to environment. Until we have penetrated a good deal deeper into the mysteries of bio-chemistry we cannot speculate on such points with any prospect of success. Meanwhile we cannot altogether rule out the possibility of the direct effect of environment in evolution. And if it has any effect it probably has a very important effect.

RECENT TEXT-BOOKS OF BOTANY

1. Fritch, F. E. and Salisbury, E. J. *An Introduction to the Structure and Reproduction of Plants*, 1920. Pp. viii and 458, with 2 plates and 230 figures in the text. G. Bell and Sons, London. Price 15s.
2. Jones, W. Neilson, and Rayner, M. C. *A Text-Book of Plant Biology*. Pp. viii and 262, with 6 plates and 36 figures in the text. Methuen and Co., London, 1920. Price 7s. 6d.
3. Small, J. *A Text-Book of Botany for Medical and Pharmaceutical Students*. Pp. x and 681, with 1350 figures in the text. J. A. Churchill, London, 1921. Price 25s.

Ten years ago it was still a reproach to British Botany that in spite of the existence of a few admirable elementary books (such as Scott's *Structural Botany*) students had to depend for their text-books mainly on translations from the German, though the study of modern scientific botany had long been securely established in this country. Today such a ground of reproach no longer exists. Recently we had occasion to review Professor Bower's *Botany of the Living Plant* (1919)¹, and the three works cited at the head of this review are excellent specimens, appearing within a few months of one another, of the activity of some of our middle aged and younger botanists in endeavouring to supply students with more or less comprehensive introductions to the study of plants.

In turning over the pages of these volumes one cannot but be impressed afresh with the enormous and ever-widening content of modern botany. At no time has the study of plants been more active and widespread; and it constantly tends to come into contact with more and more distinct departments of human knowledge and activity². At no time, certainly, has it been of more actual and potential importance to the human race. This modern development of botany has necessarily and inevitably taken place on specialised lines, resulting in the development of numerous departments of knowledge each occupying the life-time labours of many research workers. To become thoroughly acquainted with the contents of more than a very few of these departments is quite beyond the powers of any individual.

The problem of writing an adequate introductory text-book to this vast field of knowledge is thus one of considerable difficulty. In the first place the writer has to make up his mind as to what aspects of the subject it is of the greatest importance to bring most prominently before the student. If he

¹ *New Phytologist*, 18, p. 259.

² This is amusingly illustrated by the appearance on p. 191 of Professor Small's text-book, under the heading of "The Phispiral" of a reproduction of Turner's picture "Ulysses deriding Polyphemus," and on p. 394 of a diagram of the Uranium-actinium and the Uranium-radium disintegration series!

neglects this preliminary consideration he is very likely to succeed only in presenting a large number of more or less disconnected phenomena of plant life, which will tend to bewilder the mind of the reader. In an extensive work of reference such a method of treatment is perhaps justified, but in a book intended to be *read* it is fatal. In such a book the writer should set out to tell a connected story, and while the theme of this story is the same, its treatment must depend on the attitude of the writer towards plant life. From this point of view it is clear that we may have several different treatments, according as the story of plants is approached from the side, for example, of the detailed evolution of the plant world, of the physical and chemical forces at work in determining their form, structure and activities, of their relations to the actual places they occupy on the surface of the earth, or of their relations to the life of man. These aspects may of course be combined in various ways or each may serve successively as the theme of a separate section. If a choice has to be made between them we must prefer the second as the most fundamental, and after that the third and fourth. The first was dominant 20 or 30 years ago, when the tremendous stimulus to the comparative study of plant form from the standpoint of detailed evolution given by the general acceptance of the reality of the process of evolution was still fresh. Though it will always retain its interest and its vivid appeal to the imagination it must suffer from the drawback that while our knowledge of the actual causation of the variety of organic forms remains so meagre, we lack the material for a real causal treatment of the subject and tend to fall back upon teleological interpretations which are often false and always sterile.

The more fertile modes of approach are those mainly adopted in the books before us. Of these the work of Professor Fritch and Dr Salisbury must be taken in conjunction with their earlier volume, first published in 1914 and now in its fourth edition—sufficient proof of its success and popularity—*An Introduction to the Study of Plants* (Bell, 7s. 6d.). That book is an admirably clear observational and experimental introduction to botany, involving no use of the microscope and thus suitable for schools. The volume under review is intended for first year students of botany at the universities. It is divided into two parts, the first dealing with the anatomy of the seed plants and the second with the life-histories of the lower groups. The type method is abandoned, as in most of the recent books, for the sake of a more general description of the various groups, what are regarded as inessential details, which, according to the authors, have "little educational value or significance," being omitted. "Such physiology" is introduced "as is more appropriately considered in relation to microscopic structure." "Features, whether of plant-anatomy or plant-chemistry, which are of commercial importance are emphasised throughout, and it is hoped that this may serve to combat the frequent ignorance of botanical students with respect to the economic aspects of their subject." A large proportion of the illustrations are original and they are mainly taken from British or commonly cultivated plants. They are clear, well drawn and well reproduced, and demonstrate the authors' extensive first-hand acquaintance with the structural features of plants, and the careful thoroughness which they have put into the selection. The book is well written and very readable. For what it professes to be it would be difficult to improve upon, and it should be exceedingly useful to students, who will gain from its study

a clear comprehension of the nature and significance of the structural features of plants.

It would probably be a good thing to attack the question of adaptation a little more directly, even in an elementary book. The mode of exposition adopted by the authors will still, we fear, not suffice to give students clear ideas of the way in which the useful characters and powers we see in plants can originate, and the ways in which they almost certainly do not originate, though the actual statements about variation and heredity and the effect of conditions are clear, accurate and judicious enough¹. The "teleological atmosphere" is hard to escape from altogether, but several recent American writers of elementary text-books have been very successful in freeing their statements from it, for instance Dr J. G. Coulter in his *Plant Life and Plant Uses* (1913), Dr Transeau in *Science of Plant Life* (1919)—both admirable "high school texts"—and Dr Gager in *Fundamentals of Botany* (1916), which is perhaps better known in this country. None of these authors has however attempted the direct exposition we have in mind. Something rather more constructive is wanted. Prof. Small boldly adopts the theory of epimorphosis as a factor of the first importance, but the justification of this course may well be questioned.

Prof. Jones' and Dr Rayner's *Text-Book of Plant Biology* has rather a different aim. The authors point out in their preface that many students of elementary botany leave the subject before they have had an opportunity of realising its wider biological aspects, and thus miss what is often their only opportunity of acquiring that grasp of biological principles which is most desirable in the future citizen. They have therefore attempted to design an elementary course which will serve as an introduction to scientific method and enable a student to acquire an understanding of the relation of plant life to general biological knowledge.

In endeavouring to carry out these aims the authors have departed rather widely from the subject-matter of the ordinary elementary course, and though their mode of approach to the subject is exceedingly admirable in itself this fact will, we fear, tend to militate against the wide adoption of their book as a "text-book for the use of the senior classes in schools and junior classes of the University." One of the greatest obstacles to the improvement of elementary teaching is the conservatism of tradition, and the necessity under which most teachers labour of adapting their teaching to fixed examination syllabuses. We want a great deal more experiment in different methods of treatment of the subject, but it is very difficult to obtain under existing conditions. Meanwhile the multiplication of University departments in which the head is comparatively young, anxious to try new experiments in teaching, and with a nearly free hand, is all to the good.

The book under review is divided into three Parts, the first devoted to the Plant as a Machine, the second to Reproduction and the third to the Plant in Relation to the Outside World. Part I occupies nearly half the book, and contains a fairly comprehensive account of respiration, nutrition and the water relation, the treatment being essentially "biological" throughout. To each chapter there are added directions for appropriate practical work in illustration

¹ With the exception of one sentence (p. 378) which seems to imply that effective selection might take place within a pure line, though it has been stated above that this is not the case.

of the topics dealt with in the chapter. The success of one of the authors in conducting practical classes in elementary plant physiology is a guarantee of the workableness of the experiments suggested and of the practical directions given for carrying them out.

Part II contains general chapters on the process of reproduction, a very short chapter on the Outlines of Classification (the only concession to "morphology" the book contains), and another on Evolution, Variation and Heredity. This last has no directions for practical work attached. Part III consists of a chapter on Plant Response, another on Ecology and Plant Geography, and a third on the Soil.

The treatment of the subject is on the whole excellent and well calculated to fulfil the authors' primary aims. The practical drawback is that since elementary students have rarely time for more than one text-book, the omission of any treatment of morphology, which all examination syllabuses require and most teachers regard as a necessary part of the training of students, will tend to prevent the adoption of the book as a text-book in university classes.

Professor Small's book is decidedly longer, though the number of pages is considerably increased by the very numerous illustrations, and considerably more expensive than the other two. It is stated on the title-page and in the preface that it is intended primarily for medical and pharmaceutical students. The reviewer is quite clear that medical and pharmaceutical students ought not to be expected to become acquainted with so wide a range of facts and theories relating to plants as is contained in this work. It is the overloading of the syllabus in elementary biology as well as the undue preponderance of morphological detail which has been largely responsible for the desire on the part of some medical educationists to cut biology out of the syllabus altogether. This would be a deplorable action, but if it should be taken biologists will have their own unintelligent particularism largely to thank. Medical students, certainly, and probably all students who are not going to become professional botanists, should be taught not so much about plants for their own sake, but rather what plants can teach us about life as a whole. The outlook of Prof. Jones' and Dr Rayner's book is indeed much better adapted to the real educational needs of such students, though it would probably be quite impracticable to use it as a text-book in very large medical classes.

A feature of Prof. Small's book, alluded to in the preface, is the somewhat "advanced" treatment of the physico-chemical basis of physiological processes. We are inclined to agree with the author that "the average student is intelligent enough to appreciate the fundamental significance of these points, while the brighter students welcome a glimpse of what lies behind the dry bones of structural botany, and want to hear more of the inner life of the plant." At least the author's bold and straightforward method of dealing with these problems is better than the slovenly and wholly inadequate treatment which they too often receive. Another feature which will certainly be criticised is the inclusion of the author's hydrion concentration theory of geotropism, recently published in this journal, and certainly still very much "in the controversial stage." Prof. Small defends himself by saying that "in this matter these views are in the same position as many others which, in the past, have been taught dogmatically until such time as their limitations have been proved."

There is much fresh, straightforward, interesting treatment of many important topics in Prof. Small's work, but of its general plan and contents as an introduction to the subject we cannot approve. At the best it may be useful as a work of *reference* for the elementary student, but for this purpose it is hardly full enough or critical enough. We regard it as a pity that the author did not devote his remarkable gifts to the writing of a real *introduction* to botany. He has knowledge, humour, enthusiasm, courage and the power of concise yet interesting exposition, though his style, as has been said by another critic, is sometimes rather breathless. With more forethought and judgment he might, we believe, have produced the best introduction to botany that has yet been written.

The publication of these three books within a few months of one another, by heads of teaching departments, certainly does encourage the hope that the teaching of elementary botany is beginning to get into more fruitful paths.

A. G. T.

AN EXAMPLE TO BE AVOIDED.

Baines, A.E. *Germination in its Electrical Aspect*. Routledge, 1921.

Mr Baines puts down a defensive barrage in beginning the preface to his book with the following sentence:

"When this book, a plainly written account of laborious research passes into the hands of reviewers I would ask those gentlemen to remember that a great humanitarian question is involved and that while my personal opinion upon matters of detail may seem worthy of attack the fundamental truth I have put forward...is of too great importance to mankind to be passed over, or to be, to all intents and purposes, shelved by saying there is very little that is new in it." Barrages however can and must be passed through. The 'fundamental truth' which the book deals with is that the germination and growth of a plant are both governed by electrical stimuli. For example, the embryo of the dry horse chestnut seed when it falls from the tree is said to have an electrostatic charge insulated from the soil by the dry testa. When moistening occurs this charge is liberated, a current is set up and this gives the necessary stimulus to cause growth. No attempt is made by the author to examine his numerous experiments in the light of our present knowledge. In some cases the results from which he deduces evidence confirmatory of his 'truth' are explicable by well authenticated phenomena requiring no assumption of the presence of electrical currents, *e.g.* punctured acorns refused to germinate, this might be because their electrostatic charge was liberated or it might be because they had been allowed to become too dry before the experiment. In other cases the numbers of plants grown were so small that the observations are valueless, *e.g.* in one case three tufts of grass were planted out in pots and each pot was treated in a different manner. Had three thousand or even three hundred plants been taken the results might have inspired some confidence. The whole book is interlarded with the author's opinions stated without any experimental support; it also contains very numerous lengthy quotations from the works of well-known Botanists, many of which are distorted or commented on in an extraordinary fashion. In short, although this reviewer is perfectly prepared to believe in the "fundamental truth" when some sound experimental data are brought forward in its support, he has failed to find them in this book.

S. H. W.

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PERMEABILITY

By WALTER STILES

CHAPTER IV

DIFFUSION

IF two gases are separated by a partition which is subsequently removed, the molecules of one gas pass into the space occupied by the other until a condition of equilibrium is reached in which the two gases are equally distributed throughout the whole space. A similar diffusion takes place in the case of two miscible liquids, except that owing to the internal friction or viscosity the rate of equilibration is much slower. Similarly, if the two liquids brought into contact consist of solutions of different substances in the same solvent, then diffusion proceeds until the solutes are equally distributed throughout the whole of the liquid; or if the solvent contains the same dissolved substance but in different concentrations in the two liquids, diffusion proceeds until the solute is equally distributed through the whole of the solvent, always supposing that no external force is operative.

Phenomena involving the diffusion of gases do indeed occur in the plant, as in the processes of carbon-assimilation, respiration and transpiration. But directly any gas reaches the surface of a cell any further passage of the molecules of the gas through the cell takes place in an aqueous medium, and in problems of permeability it is with diffusion through a liquid that we are concerned, and in most cases with diffusion through an aqueous medium.

The first systematic researches on diffusion of liquids were made by Thomas Graham (1851), who investigated the diffusion of a variety of substances dissolved in water. He showed that the quantity

of salt diffusing through unit area of the solvent depended on the concentration of the salt, on the nature of the salt, and on the temperature. The greater the difference of concentration, the more salt diffused in unit time, and the higher the temperature the faster the rate of diffusion.

The subject was later investigated by Fick (1855) who, by comparison of the problem with that of the conduction of heat worked out by Fourier, propounded the well-known equation which has come to be known as Fick's law. This equation is

$$dC = -D \frac{\partial c}{\partial x} dt,$$

where C is the quantity of salt passing through unit area in a time dt at a point x where the concentration gradient (*i.e.* the rate of change of concentration with distance) is $\frac{\partial c}{\partial x}$. The value of D is constant for any particular substance in any definite concentration and at a definite temperature and is called the coefficient of diffusion for the substance, or the diffusivity. It is thus the quantity of salt diffusing across unit area in unit time when the concentration gradient is unity (that is, when two cross-sections of the liquid at unit distance apart differ by unity in their concentrations).

Fick's law states therefore that the quantity of salt diffusing across unit area is proportional to the coefficient of diffusion, to the concentration gradient and to the time of action. This law has been abundantly verified since the time of Fick. For the solution of the differential equation reference may be made to the work of Fourier (1822, 1878) or to the various more modern works dealing with Fourier's equations (for example, Carslaw, 1906; Weber and Riemann, 1910-1912). Reference may also be made to a paper by Kelvin (1889, 1890) in which are given the curves showing the relation between concentration of solution at a point distance x from the initial surface of contact between water and a saturated solution, the position of the point x , and the time of action.

It will be sufficient here to consider only the simplest case, that of a salt diffusing into a cylindrical column of water. If we regard the column of water as infinitely long, the solution of Fourier's equation for this case is

$$u = u_0 \left\{ 1 - \frac{2}{\sqrt{\pi}} \int_0^q e^{-q^2} dq \right\},$$

where

$$q = \frac{x}{2\sqrt{Dt}},$$

and u is the concentration at a point in the cylinder distant x from the initial surface of contact between water and the solution, and after a time t has elapsed from the commencement of diffusion, u_0 is the concentration of the original solution at the mouth of the cylinder and D is the coefficient of diffusion. The value of

$$\frac{2}{\sqrt{\pi}} \int_0^q e^{-q^2} dq$$

for different values of q is given in tables of the probability integral (see, for example, Peirce, 1910).

It follows from this equation that if u is constant, that is, if we consider the march forward into the column of water of a particular concentration of diffusing substance, that q must also be constant. Hence

$$\frac{x}{2\sqrt{Dt}} = \text{constant},$$

or, stated in words, the distance any particular concentration of salt has reached is proportional to the square root of the time of action and to the square root of the coefficient of diffusion. This relation has been repeatedly verified (Coleman, 1887, 1888; Chabry, 1888; Voigtländer, 1889).

Various methods have been devised for the measurement of coefficients of diffusion. These may be classified as follows:

(1) Those depending on the estimation after a certain time by chemical analysis of the concentration of the solution occupying different layers in the vessel in which diffusion is proceeding.

(2) Methods in which the concentration of different layers of solution is determined from estimations of the density of the solution.

(3) Methods based on the estimation of the rate at which substances go into solution.

(4) Indicator methods in which the presence of an indicator in the liquid shows when a certain concentration of the diffusing substance is reached at any particular place.

(5) Electrical, and (6) optical methods.

For a description of these various methods reference may be made to the article on Diffusion by Waitz (1908) in Winkelmann's *Handbuch der Physik*. An electrical conductivity method is described by Haskell (1908).

In Table IV are given the coefficients of diffusion of a number of substances in water. These values are taken from the results of Thover (1901, 1902), Scheffer (1888), Schuhmeister (1879) and Heimbrod (1903, 1904), and the calculations made by Stefan (1879) from the data of T. Graham.

TABLE IV
Coefficients of Diffusion of Various Substances

Substance	Concentration in gm.-mols. per litre	Tempera- ture in °C.	Coefficient of Diffusion in $\frac{\text{cm.}^2}{\text{sec.}} \times 10^{-5}$	Observer
HCl	3.2	19.2	4.50	Thovert
	0.02	19.2	2.45	"
HNO ₃	3.9	19.5	2.85	"
	0.02	19.5	2.45	"
H ₂ SO ₄	2.85	18.0	1.85	"
	0.005	18.0	1.51	"
KOH	3.9	13.5	2.81	"
	0.02	13.5	1.95	"
NaOH	3.9	12.0	1.14	"
	0.02	12.0	1.30	"
KNO ₃	3.9	17.6	1.03	"
	0.02	17.6	1.48	"
KCl	2.95	17.5	1.85	"
	0.02	17.5	1.57	"
NaCl	3.9	15.0	1.18	"
	0.02	15.0	1.09	"
CaCl ₂	1.22	9.0	0.83	Scheffer
	0.0468	9.0	0.79	"
K ₂ SO ₄	1.5	10	0.87	Schuhmeister
	0.95	19.6	0.92	Thovert
ZnSO ₄	2.95	19.5	0.38	"
	0.025	19.5	0.58	"
MgSO ₄	3.23	10	0.31	Scheffer
Ethyl alcohol	3.75	11	0.52	Thovert
	0.05	11	0.85	"
Glycerin "	1.75	10.14	0.35	Heimbrodtt
	0.125	10.14	0.41	"
Citric acid	0.0303	4.5	0.39	Scheffer
Sucrose	1.97	18.5	0.15	Thovert
	0.97	18.5	0.28	"
Albumin	—	13	0.073	Graham-Stefan

From the numbers given in this table it will be observed that the coefficient of diffusion varies considerably for different substances. It is also dependent upon the concentration of the diffusing substance, upon the temperature and upon the nature of the medium through which diffusion is taking place. In the following discussion, except where expressly stated otherwise, the medium is always assumed to be water.

As regards the influence of the nature of the substance on the coefficient of diffusion, generally speaking the diffusivity is high for electrolytes and substances of low molecular weight, while it decreases with increasing complexity of the molecule, being lower for substances like sugars and very low for colloidal substances. Of the commoner electrolytes, acids and alkalies diffuse most rapidly, and

in general electrolytes with the highest diffusivity have the highest electrical conductivity.

Mathematical formulae have been evolved to express these relations in the case of diffusion of non-electrolytes. Thus Sutherland (1905) and Einstein (1905, 1906) derived the formula

$$D = \frac{RT}{N} \cdot \frac{1}{6\pi\eta\rho},$$

where D is the coefficient of diffusion, R is the gas constant, T the absolute temperature, N the Avogadro constant (that is, the number of molecules in one gram molecule), η the viscosity of the solvent, and ρ the radius of the diffusing molecules which are assumed large in comparison with those of the solvent. Sutherland showed, however, that should the molecules of the solvent be large in comparison with those of the solute, the relation more nearly approximates to

$$D = \frac{RT}{N} \cdot \frac{1}{4\pi\eta\rho}.$$

Von Smolukowski (1906) obtained a similar expression but with a different constant, his formula being

$$D = \frac{64}{27} \cdot \frac{RT}{N} \cdot \frac{1}{6\pi\eta\rho}.$$

A general confirmation of this formula experimentally has been made by Svedberg and Andreen-Svedberg (1909, 1911) who could not, however, decide whether the constant in the equation of Einstein, Sutherland and von Smolukowski is 1 or 64/27.

The relation between molecular size and coefficient of diffusion has been stated in another form by Exner (1867, 1874, 1877) for the case of gases. Exner's conclusion is expressed by the equation

$$D\sqrt{M} = k,$$

where D is the coefficient of diffusion, M the molecular weight and k a constant. This relation has been extended to the case of non-electrolytes by Öholm (1910), who confirmed it experimentally for a number of sugars and other substances, and who used it to determine the molecular weight of dextrin from the coefficient of diffusion of the latter. A third formula has been proposed by Herzog (1910), namely

$$D\eta\sqrt[3]{Mv} = \text{constant},$$

where v is the specific volume and the other symbols have the signification already assigned to them. This relation is stated to hold for a number of non-electrolytes (Padoa and Corsini, 1915).

There seems thus good reason for concluding that the coefficient of diffusion is related in inverse fashion to the complexity and magnitude of the molecule.

With regard to the connection between coefficient of diffusion and electrical conductivity, Nernst (1888) put forward the following equation to express the relation between diffusivity and ionic mobilities:

$$D = RT \cdot \frac{uv}{u + v},$$

where u and v are the ionic mobilities of anion and cation, and the other symbols have the same signification as before. Nernst himself realised that this formula could only be approximately correct as the diffusivity varies with concentration, nor does the formula take into account the diffusion of the undissociated part of the molecule.

To allow for the influence of concentration on diffusion, Wiedenburg (1899) modified Nernst's formula as follows:

$$D_c = RT \left(\frac{2uv}{u + v} - w \right) \left(1 + \frac{4C}{K} \right)^{-\frac{1}{2}} + w,$$

where D is the coefficient of diffusion at a concentration c , K the dissociation concentration at the concentration and temperature in question, and w the molecular mobility.

Arrhenius (1892) obtained good agreement between experiment and the equation

$$D_c = D_\infty \left(1 + \frac{2BC}{RT} \right) \left(1 - \frac{\alpha C}{2} \right)^2,$$

where D_∞ is the coefficient of diffusion at infinite dilution, α a constant depending on the viscosity, and B a constant.

Arrhenius found the coefficient of diffusion of hydrochloric acid and of potassium hydroxide increased with increasing concentration, but that the reverse was the case with acetic acid, sodium hydroxide and sucrose. Scheffer also found an increase of diffusivity with increasing concentration in the case of hydrochloric acid; with sodium and potassium chlorides he found no notable change, while with sodium nitrate, sodium thiosulphate and silver nitrate the diffusivity diminishes with increasing concentration. A careful investigation of the influence of concentration on diffusivity of a number of electrolytes has been made by Öholm (1905); the data in Table V are taken from among his results. Data with regard to the influence of concentration on diffusion of glycerin, urea, sodium chloride and hydrochloric acid are given by Heimbrodt (1903, 1904).

TABLE V
Influence of Concentration on the Diffusivity at 18° C.
(Data from Öholm)

Concentration in gram-equivalents per litre	Coefficient of Diffusion in $\frac{\text{cm.}^2}{\text{sec.}} \times 10^{-5}$			
	NaCl	KCl	HCl	KOH
0.01	1.35	1.69	2.69	2.20
0.02	1.33	1.66	2.64	2.19
0.05	1.32	1.63	2.61	2.17
0.10	1.29	1.61	2.58	2.15
0.20	1.27	1.58	2.55	2.13
1.00	1.24	1.54	2.57	2.15
2.00	—	1.53	—	2.19
2.8	1.23	—	—	—
3.6	—	1.55	—	—
5.5	1.23	—	—	—

That the rate of diffusion of substances increases with rise of temperature was observed by Graham. Since these early observations quantitative determinations on the effect of temperature on the diffusivity have been made for a number of substances. Weber (1879) found that for every rise in temperature of 1° C. the coefficient of diffusion of zinc sulphate in water increased by about 0.026 of its value at 18° C. Later Seitz (1898) confirmed this value and obtained a similar one (0.024) for zinc acetate. A number of substances were examined by de Heen (1884) at temperatures varying from 15° to 60° C. In Table VI are shown the values he obtained for the coefficient of diffusion at any temperature θ , this being measured in centigrade degrees distant from 60° C. taken as zero, and reckoned as positive *downwards* from 60° C.

TABLE VI
Influence of Temperature on the Coefficient of Diffusion
(Data from de Heen)

Substance	Coefficient of Diffusion in $\frac{\text{cm.}^2}{\text{sec.}} \times 10^{-5}$
MgSO ₄	0.85 (1 - 0.0119 θ)
KNO ₃	3.07 (1 - 0.0127 θ)
NaCl	2.72 (1 - 0.0121 θ)
Na ₂ HPO ₄	2.06 (1 - 0.0128 θ)
K ₂ CO ₃	1.63 (1 - 0.0127 θ)

It will be noticed that the relative increase in diffusion for the same rise in temperature is almost independent of the substance

among those examined, although the absolute coefficient of diffusion varies greatly. These results agree well with those obtained for zinc salts to which reference has already been made.

On theoretical grounds Nernst (1888) propounded the formula

$$D_{\theta} = D_{18} [1 + \alpha (\theta - 18)]$$

to express the influence of temperature on the coefficient of diffusion, D_{θ} and D_{18} being the coefficients of diffusion at θ° C. and 18° C. respectively, and α a constant having the value 0.026 for neutral salts and 0.024 for acids.

Assuming the correctness of Nernst's general formula, the value of the temperature coefficient α in Nernst's equation has been calculated by Öholm from measurements of the coefficient of diffusion of a number of electrolytes (Öholm, 1902, 1905) and non-electrolytes (Öholm, 1910) at temperatures between 0° and 20° C. The values found by him are summarised in Table VII.

TABLE VII
Temperature Coefficient of Diffusivity of a number of substances
(Data from Öholm)

Substance	Temperature Coefficient
HCl	0.019
NaCl	0.025
KCl	0.0235
LiCl	0.027
KI	0.0235
NaOH	0.023
KOH	0.021
Acetic acid	0.028
Sucrose	0.032
Lactose	0.032
Maltose	0.032
Raffinose	0.032
Arabinose	0.044
Dextrin	0.035
Nicotine	0.016

Although there are obvious exceptions, Öholm concludes that in general the temperature coefficient of diffusivity is less the greater the diffusivity. Thus hydrochloric acid, with its very high coefficient of diffusion, has a very low temperature coefficient of diffusivity, while the slow diffusing sugars have temperature coefficients considerably higher than those of neutral inorganic salts.

It should be noted that it is assumed by all workers that the relation between diffusivity and temperature is a linear one. While this may be approximately true over the small temperature intervals with which Öholm worked, it is scarcely likely to be an exact

representation of the facts over a wide temperature interval; it may be sufficiently accurate over the temperature range with which we are generally concerned in living organisms.

We have so far considered the laws of diffusion in a medium which at equilibrium is homogeneous. In the organism, as we have seen, we have to deal very largely with heterogeneous systems. As the simplest case of a heterogeneous system we may consider two immiscible liquids separated by a phase boundary. That such systems actually occur in the living cell there can be little doubt. If a solute is soluble in both the liquids, its distribution when diffusion has proceeded to equilibrium will not be uniform throughout the system; on the contrary, the solute generally distributes itself unequally between the two solvents. This phenomenon was investigated by Berthelot and Jungfleisch (1869-1872) and later by Nernst (1891). It is found that if a substance has the same molecular complexity in the two solvents the ratio of the concentration of the solute in one solvent to its concentration in the other is a constant whatever the concentration. Thus if c_1 is the concentration in one solvent and c_2 the concentration in the other solvent,

$$\frac{c_1}{c_2} = K,$$

where K is a constant and called the *partition coefficient* or the *distribution ratio*. If several solutes are present together each one distributes itself between the solvents according to its own partition coefficient independently of the others; that is, K is independent of the presence of other solutes.

If the solute should undergo polymerisation in one of the solvents, the partition law becomes modified to

$$\frac{c_1}{c_2^{1/n}} = K,$$

where n represents the number of molecules associated together in one of the solvents.

With the law of partition coefficients may be compared the law governing the distribution of a substance between a solvent and an adsorbent at equilibrium. The quantity $\frac{x}{m}$ in the adsorption equation is of the nature of a concentration, and if for this we write c_1 , and if for the concentration of the solute at equilibrium we write c_2 , the adsorption equation becomes

$$\frac{c_1}{c_2^{1/p}} = K,$$

which represents the distribution of the solute between solvent and adsorbent. The similarity of this equation to that of the partition law is obvious.

The principle involved in the partition law has been applied with great success to the separation of substances which otherwise have so far been found inseparable, or are only separable with great difficulty. The separation of the four pigments of the green leaf by Willstätter (1913) is almost entirely based on the different partition coefficients of the four pigments and the substances with them, in the commoner organic solvents.

It is now necessary to consider how far the laws governing the rate of diffusion are modified in more complex systems.

The case of an electrolyte diffusing in a solution of another substance instead of in pure water has been investigated by Öholm (1912). He found that the presence of a non-electrolyte such as glycerin or sugar appreciably reduces the rate of diffusion. In the following table are given the values obtained by Öholm for the coefficients of diffusion of potassium chloride in solutions of non-electrolytes of different concentrations.

TABLE VIII
Diffusion of Potassium Chloride in Solutions of Sucrose and
Glycerin of Various Concentrations
(Data from Öholm)

Substance	Concentration in gram-mols. per litre	Coefficient of Diffusion at 18° C. in $\frac{\text{cms.}^2}{\text{sec.}} \times 10^{-5}$
Sucrose	0.0	1.535
"	1.5	0.486
"	2.0	0.255
Glycerin	5.0	0.501
"	7.48	0.201

We now come to the important question of the diffusion of substances in colloidal systems. The diffusion of various substances in gels has received a considerable amount of attention, partly because of its obvious bearing on physiological problems (cf. Chapter II), and also because it lends itself more readily in some ways to investigation than diffusion in free liquids.

It was concluded by Graham (1861) that sodium chloride diffused as rapidly through gelatine as through water. De Vries (1884) on the other hand concluded from observations of the colour that

potassium chromate diffuses more slowly through 4 per cent. gelatine than through water, and Coleman (1888), examining the diffusion of a number of electrolytes in gels, also concluded that the presence of the jelly brings about a retardation in the rate of diffusion. Hagenbach (1898) came to a similar conclusion in regard to the diffusion of gases in gelatine. Henri and Calugareanu (1903), from observations on the diffusion of a number of dyes in water and 1, 3 and 5 per cent. gelatine, concluded that the rate of diffusion was the same through the gelatine as through water if the former had not set to a gel, but that if this had taken place, the rate of diffusion was slower. Bechhold and Ziegler (1906) concluded that electrolytes and non-electrolytes diffuse through gelatine and agar gels more slowly than through water.

Öholm (1913) compared the diffusion of potassium chloride from a normal solution into gels of 2, 5 and 10 per cent. gelatine. His results, summarised in the accompanying table, indicate very clearly the retarding effect on diffusion of the gelatine.

TABLE IX
Diffusion of Potassium Chloride from a Normal Solution into
Gels of Gelatine of Various Concentrations
(Data from Öholm)

Concentration of Gelatine in per cent.	Kind of Gelatine	Coefficient of Diffusion at 20° C. in $\frac{\text{cm.}^2}{\text{sec.}} \times 10^{-5}$
2	Kahlbaum (golddruck)	1.52
5	" "	1.44
10	" "	1.31
2	A brand from Dundee	1.50
5	" " "	1.41
10	" " "	1.28

If these numbers are compared with those given in the preceding table it will be observed that, as Öholm points out, a gelatinous body affects the diffusion of an electrolyte in just the same way as any other non-electrolyte. Unpublished observations of Stiles and Adair indicate that the presence of gelatine decreases the rate of diffusion of sodium chloride progressively with increase in concentration of the gelatine. There can be no doubt that the coefficient of diffusion of electrolytes is less in gels of gelatine than in water.

Voigtländer (1889) made a study of diffusion in agar-agar gels varying in concentration from 1 to 5 per cent. and found that the

rate of diffusion was practically independent of the concentration of the gel. Sodium chloride, magnesium chloride and hydrogen chloride were among the substances examined, but the unpublished researches of Stiles and Adair have failed to confirm this conclusion in the case of sodium chloride, which appears to diffuse more slowly the greater the concentration of agar-agar, so that agar-agar behaves in the same way as gelatine in reducing the rate of diffusion.

The consequence of Fick's law in the case of linear diffusion in one direction, namely that $x = C\sqrt{Dt}$ where C is a constant (see p. 139), has been confirmed for diffusion into gels by Coleman (1888), Chabry (1888), Voigtländer (1889), von Fürth and Bubanović (1918), Stiles (1920) and Adair (1920), these various authors using a great variety of diffusing substances and gels of gelatine, gelose and agar-agar. The law holds equally well when the gel contains an indicator to mark the progress of a layer of definite concentration provided the concentration of the indicator does not approach too near to that of the diffusing substance (Stiles, 1920).

There are few observations on the influence of temperature on the coefficient of diffusion in gels. The results of Voigtländer on diffusion in agar-agar gels indicate a greater increase in diffusivity brought about by an increase in temperature from 20° C. to 40° C. than that brought about by an increase from 0° C. to 20° C. Voigtländer employs the equation

$$D_{\theta} = D_0 (1 + \beta\theta)^2$$

to express the relation between the coefficient of diffusion and temperature, D_{θ} and D_0 being the coefficients of diffusion at θ° C. and 0° C., and β a constant. It will be observed that as the values of β are small (0.01 to 0.02) in comparison with unity, when θ is also small then approximately

$$D_{\theta} = D_0 (1 + 2\beta\theta),$$

since the term $\beta^2\theta^2$ is small in comparison with $1 + 2\beta\theta$. Consequently, when θ is small the relation between the temperature and the coefficient of diffusion is approximately a linear one as assumed by de Heen, and by Öholm following Nernst. But when θ is large the term $\beta^2\theta^2$ is not negligible, and the coefficient of diffusion increases more rapidly with rise in temperature the higher the temperature. The unpublished results of Stiles and Adair on the diffusion of sodium chloride in agar-agar gels at temperatures from 0° C. to 40° C. support this latter assumption rather than the opinion of a linear temperature coefficient.

The observations of Chabry (1888) show that the course of diffusion of an electrolyte through a dead animal tissue is similar to its diffusion through a gel. Prismatic pieces of hyaline cartilage stained with orcein were fitted into a tube and any space between the tissue and the tube were filled with a transparent oil. The penetration of acid into the tissue was then followed by the indicator method in the same way as in the case of a gel, and it was found that the consequence of Fick's law, that $\frac{x}{\sqrt{t}} = \text{constant}$, held in this case as well as in the cases of diffusion in water and in gels. The value of the constant was however found to be considerably less than with diffusion into a gel.

The diffusion of crystalloids through colloidal systems such as gels and dead organic tissue apparently proceeds in the same way as through water, although less rapidly. But the diffusion of colloids differs strikingly in a simple liquid and in a colloidal system. It is well known that Graham drew the fundamental distinction between colloids and crystalloids on account of the non-diffusibility of the former through membranes of parchment and other colloidal materials, while crystalloids diffused readily through such membranes. The diffusibility or non-diffusibility of a substance or group of substances through membranes is however generally not an absolute property of the membrane; it is a matter of degree. Thus colloidal membranes which are permeable to crystalloids do not let them all through equally readily. This is a matter of obviously great importance in relation to permeability of living tissue and requires to be considered in some detail; it therefore forms the subject of the next chapter.

(To be continued.)

A STUDY OF SOME OF THE FACTORS CONTROLLING THE PERIODICITY OF FRESHWATER ALGÆ IN NATURE

By WILLIAM J. HODGETTS, M.Sc.

(With 11 figures in the text)

I. INTRODUCTION

THIS paper deals with the periodical phenomena of the algal vegetation of a small freshwater pond. The research was undertaken not only for the purpose of elucidating the periodicity of the chief algal species present, but also with the object of demonstrating what dependence, if any, exists between the development and reproduction of the various Algæ, and the meteorological and other conditions, special attention being paid to the part played by the concentration of the water. Throughout the paper the principle of limiting factors¹ has been employed in discussing the influence of these various external conditions upon the growth and reproduction of the Algæ of the pond. The pond chosen is situated near Hawkesley Hall (and thus in the sequel is termed Hawkesley Hall Pond), 1½ miles from King's Norton, near Birmingham, and the observations extend over a period of 3½ years, namely from January 1918 to June 1921, and are based upon numerous samples collected at monthly (at times fortnightly) intervals.

The pioneer work on algal periodicity, as observed in small ponds, was commenced in this country by F. E. Fritsch (13, 14), and later Fritsch and Rich published a series of "Studies on the Occurrence and Reproduction of British Freshwater Algae in Nature" (16, 17, 18). Many of the conclusions arrived at by these workers have been confirmed by the present writer in the observations recorded below. Other contributors to the study of algal ecology in this country are G. S. West (31), and also W. and G. S. West (32, 33, 34), although the papers quoted of the latter authors deal mainly with the periodicity of the phytoplankton of British lakes. The comparatively recent account of the occurrence of freshwater Algæ in nature by the late Prof. G. S. West (31, pp. 418-451) is a useful though necessarily short *résumé* of our knowledge of the subject up to 1916.

B. M. Griffiths (19) has studied the algal conditions of a pool near

¹ A principle first utilized in algal ecology by Fritsch and Rich (16, 17, 18).

Kidderminster, and E. M. Delf (12) has given an account of the algal periodicity observed in ponds on Hampstead Heath; while continental workers on the periodicity of freshwater Algæ are Comère (6, 7, 8), Rabanus (26), and also Pevalek (24). Reference will not be made to all the workers who have investigated experimentally the relation between the growth of Algæ and various cultural conditions, as many of these artificial conditions are never realised in nature—although, as pointed out by Fritsch (14), they may be paralleled.

The chief American worker on the subject is E. N. Transeau (27, 28), who has published results based on $7\frac{1}{2}$ years continuous records of the algal conditions in Central Illinois; while other American contributors are H. B. Brown (4), Copeland (9), Platt (25), and more recently Andersen and Walker (1). Various workers in America have investigated the relation between algal growth and the dissolved gases of the water, and in this connection reference may be made to the interesting papers of Birge and Juday (3), and Chambers (5), further literature being cited by these authors.

It has not been thought necessary in a paper of this sort to refer to all the numerous workers who have contributed to our knowledge of the freshwater phytoplankton, since this constitutes rather a special branch of algal biology, requiring methods of attack somewhat different from those adopted in the present line of research; but papers on the freshwater plankton are occasionally referred to in what follows. As pointed out by Fritsch and Rich (18), and West (30), the smaller pieces of water are alone suitable for demonstrating the relations between external conditions and algal growth, since larger bodies of water react much more slowly to variations in the meteorological and other conditions than do ponds and small bodies of water generally.

II. GENERAL ACCOUNT OF HAWKESLEY HALL POND AND ITS PHANEROGAMIC FLORA

Hawkesley Hall Pond is situated in a meadow about $\frac{1}{4}$ mile N.W. of Hawkesley Hall, and is not at all large, its area, when the height of the water is at a maximum, being roughly 300 sq. metres, while the greatest depth, although not known exactly, is probably between $1\frac{1}{2}$ and 2 metres. It is an irregular triangle in shape (cf. Fig. 1), with the marginal banks for the most part rather low, and almost all round the pond bearing a thick growth of rushes, together with other Phanerogams (see below). There are no trees at the margin of the pond, the nearest being a few oaks by the fence (shown in Fig. 1)

on the west side of the pond. These trees, however, could cast only a feeble shade over the extreme west side of the pond during the evenings¹. The pond is thus in a very exposed, unshaded locality,

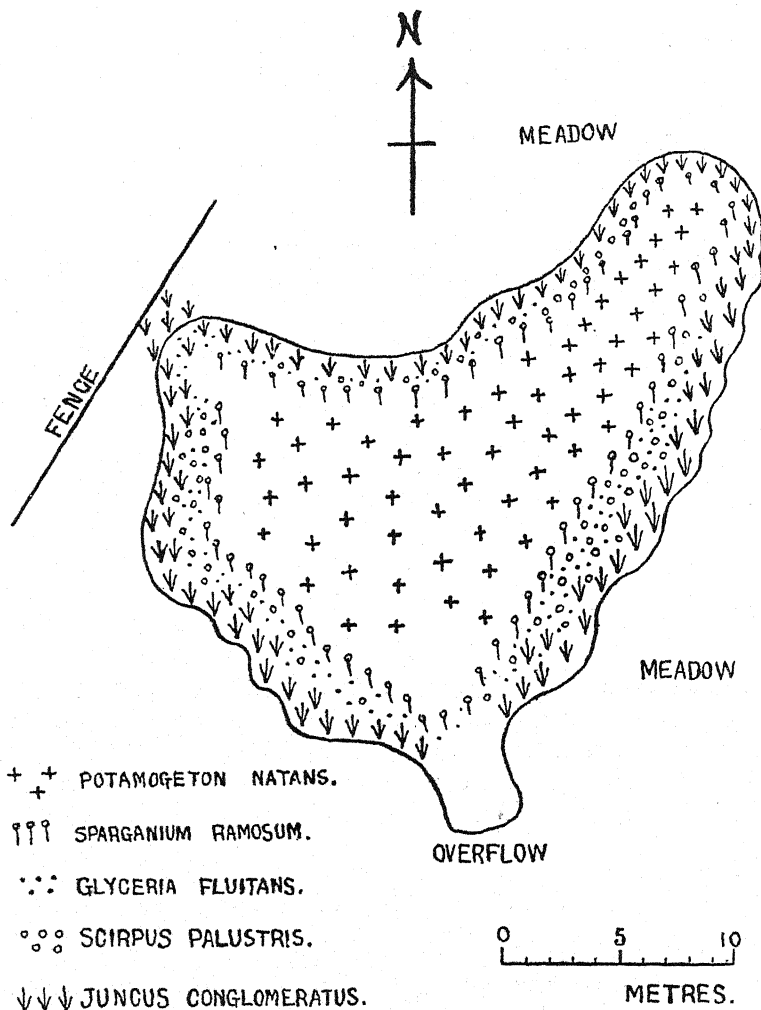


Fig. 1. Rough sketch-map of Hawkesley Hall Pond, showing the distribution of the Phanerogamic Vegetation in July 1920.

receiving practically the full force of the sun and wind, and consequently is rapidly affected by the varying meteorological conditions.

¹ A few low hawthorn bushes are present on the N.W. side of the pond,* but they do not cast any shade over the pond.

On the extreme west side of the pond is a patch of marshy ground, passing under the fence, and by means of which the pond, during times of very high water, is put in temporary communication with another smaller pond (not shown in Fig. 1) located on the opposite side of the fence. This second pond is very much shaded by trees, and has not proved very suitable for a study of algal periodicity; in the present paper it is not again referred to.

The soil of the district overlies Keuper Marl, and round the pond is of the nature of a rather marly clay, which is very impervious when wet. The general surface of the meadow slopes upwards towards the pond, which is situated at the highest point of this particular field, but towards the north-west the ground rises gently again, and it is mainly water which drains off the land from this direction which feeds the pond. Since the latter never completely dries up even during the hottest weather—although at such times it contracts in volume considerably—it seems likely that there may be a bottom-spring which to a certain extent supplies the pond, but this point was not definitely settled. When flooded with heavy rain the water overflows at the shallow south corner, and drains off down the sloping meadow, so that it is impossible for the water-level to exceed a certain maximum height, which is determined of course by the height of the ground at the southern overflow corner. The pond is some distance from a road, and no field-path runs near it, and on the whole it is allowed to remain comparatively undisturbed, which to some extent may account for the richness of its algal flora.

During the summer months the pond supports an abundant phanerogamic flora, which exhibits a more or less zonal arrangement round the pond (Fig. 1). Four well-marked zones can be recognised generally throughout the summer, these being, commencing with the outermost:

- (1) a zone of *Juncus conglomeratus*;
- (2) a zone of *Scirpus palustris* and *Glyceria fluitans* (these species generally co-dominant);
- (3) a zone of *Sparganium ramosum*; and
- (4) a central area of *Potamogeton natans*.

The marginal zone of rushes is well-marked and extends all round the pond, with the exception of the south overflow corner, covering the damp banks just above water-level. *Juncus conglomeratus* being a markedly social species it is generally easily able to exclude competitors, but a few marsh-loving plants (e.g. *Galium palustre*) are sometimes present.

The second zone of *Scirpus palustris* and *Glyceria fluitans* is rather a mixed one, but for the most part these two species are co-dominant, although in places *Scirpus palustris* alone is dominant. *Alisma Plantago* occurs sporadically. This zone is absent altogether in places, while it rarely extends into water deeper than 20 cm.

The third zone (of *Sparganium*) is very well-marked and extends practically all round the pond (see Fig. 1), and varies in width from 45 to 120 cm.—the latter where the slope of the bottom is very gradual, as on the east side of the pond.

All the central region of the pond is occupied by the *Potamogeton nutans*—a plant which has an important influence upon the algal vegetation of the pond, as will be seen later. During the winter and early spring (November to March) the *Potamogeton* is dormant, but development commences usually in early April, and by the end of June, or in early July, a great part of the water-surface of the pond is covered by the floating leaves of this plant, the spaces between the leaves being generally occupied by floating masses of filamentous Algæ. It flowers in June and July. In September its leaves gradually decay, while by the end of October all the leaves are dead, many being free and decaying in the water.

The pond is very similar in its phanerogamic vegetation to those described by Walker⁽²⁹⁾, at Bramhope, near Leeds, all the zones of vegetation (except the second) occurring in Hawkesley Hall Pond being represented in the Bramhope ponds. Doubtless ponds of this type, with similar or nearly similar phanerogamic plants, are frequent in many parts of the country; they are certainly not uncommon in the midland counties.

III. METEOROLOGICAL DATA

The more important meteorological data for the purpose of this study are those of temperature, rainfall, and bright sunshine. At the time of collecting the monthly samples of Algæ the temperature of the water and of the air near the pond were recorded, but these are not given here, since they have been found to emphasise rather too much merely temporary and often very fleeting temperature-conditions which by chance obtained at the time the samples were being collected. Of the three temperature-curves given in the lower part of Fig. 2 the uppermost gives the average maximum temperature of the air, and the lowest the average minimum for each month, while the middle curve gives the mean of these two; the data being taken from the official Monthly Weather Reports (Birmingham

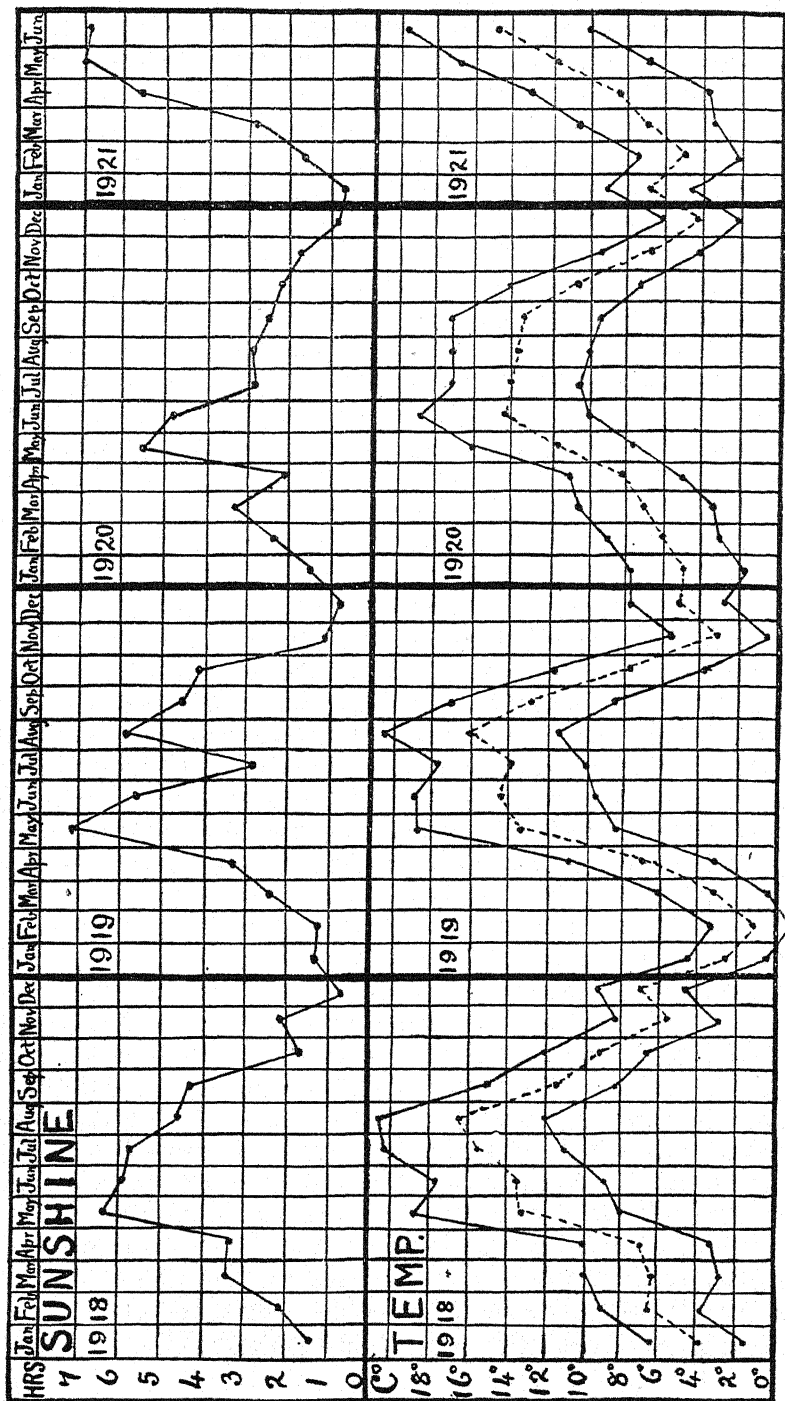


Fig. 2. The topmost curve shows the daily mean, in hours, of bright sunshine for each month. Of the three temperature-curves in the lower half of the figure the uppermost gives the average maximum temperature of the air (in degrees Centigrade), the lowest curve the average minimum, for each month, while the middle broken line represents the mean of these two. All the data are from the Monthly Weather Reports (Birmingham Station).

Station)¹. The average temperature-conditions of the air in the neighbourhood of the pond probably did not differ in any important degree from those which obtained at the Birmingham Observatory.

The sunshine-data (the daily mean, in hours, of bright sunshine for each month) are plotted in the upper part of Fig. 2, while the monthly rainfall (in mms.) is represented in the upper part of Fig. 3, the records in both cases also being taken from the Monthly Weather Reports (Birmingham Station). These curves will frequently be referred to in the detailed discussion of the occurrence of the various algal species which will follow, but several noteworthy points in connection with the weather during the period of observation may be mentioned here. The years 1918 and 1919 were fairly normal, but 1920 was a very abnormal year. The summer of 1920 was characterised by a low temperature, and it was deficient in sunshine; while the winter of 1919-1920 was very mild. In the case of each of the three complete years the sunniest month was May, and the dullest month December.

IV. CONCENTRATION OF THE WATER

With regard to the variations of the water-level of the pond, shown in Fig. 3, the information was obtained from a convenient wooden stake, driven into the bottom of the pond at a short distance from the margin. When the water was at its maximum height a definite length of the stake projected above water, while even at the lowest ebb noted during the period of observation the stake was never completely out of the water, so that by measuring the length of stake above the water, each time the pond was visited, a record of the fluctuation of the water-level was obtained. Of course this method gives far more exact information than vague terms such as "high," "rather low," etc., such as several workers in this connection have used.

It was recognised at the commencement that periodical chemical analysis of the water of the pond would have given data probably of great value in elucidating the various factors controlling the development of the various algal species, but at the time the work was commenced the writer was not in a position to carry out complete chemical analyses. As a substitute the total amount of dissolved matter in the water was determined each month (from June 1918). This was obtained by carefully evaporating to dryness, in a porcelain evaporating dish of known weight, 100 c.c. of the filtered pond-water

¹ Monthly Weather Report of the Meteorological Office, London.

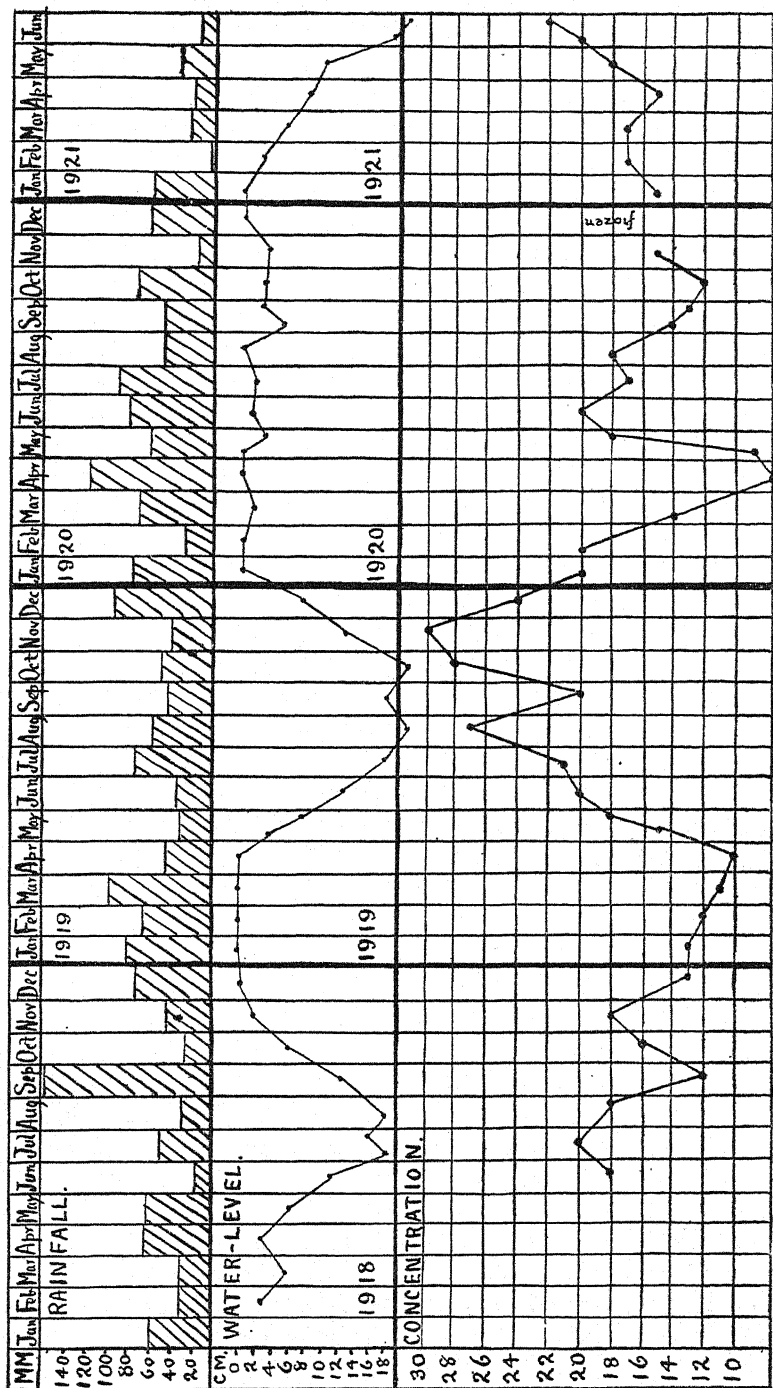


Fig. 3. The rainfall-data at the top of the figure give the monthly rainfall in millimetres (data from Monthly Weather Reports, Birmingham Station). The middle curve shows the height of the water of Hawkesley Hall Pond for each month (in centimetres) below the maximum height attainable. The lowest curve represents the variation in the total amount of dissolved matter in the water of Hawkesley Hall Pond, the figures being "degrees" (parts per 100,000 of water). See Section IV for further details.

(obtained at the time the monthly samples were collected) over a steam-bath and weighing the dish with its residue¹.

It will be seen on reference to the lowest curve of Fig. 3, where the results, in parts per 100,000 parts of water (in the sequel termed "degrees")², are plotted, that the total dissolved matter in the water varied during the $3\frac{1}{2}$ years between rather wide limits, namely between 8 and 30 degrees. A comparison of this concentration-curve with the curve showing the fluctuations in the water-level, and also with the rainfall data (all given in Fig. 3), reveals several interesting and somewhat unexpected results. In the first place the lowest concentration in both 1919 and 1920 was reached in the month of April, a result of the dilution of the pond by the winter and early spring rains. As one would rather expect, the two curves of Fig. 3 to a great extent vary inversely, the periods of low water-level generally corresponding to high concentrations, and *vice versa*, this being very obvious in the latter half of 1919; but this is not always the case, and in the autumn months especially the water-level is often rising at the same time as the concentration of the water is increasing.

Thus from September 21st to October 19th, 1918, the water rose 6.2 cm. but the dissolved matter in the water between the same dates increased from 12 to 16 degrees; and notwithstanding a further rise in the water-level of 3.8 cm. between October 19th and November 16th the concentration had increased to 18 degrees by the latter date. Again from October 18th to November 22nd, 1919, the level of the water rose 8 cm., but the dissolved matter of the water also increased from 28 to 30 degrees. During the autumn of 1920 the phenomenon of the concentration increasing with a rising water-level was not shown so well, since the water-level was fairly constant between September and November, but even so the concentration showed a decided increase between October and November.

It is obvious, therefore, that a correct idea of the variation of the concentration of the water cannot be obtained merely from an examination of the fluctuations of the water-level of a pond, and

¹ The same dish was used throughout, and it was kept specially for this purpose, never being used for any other work. After evaporating the water to dryness the dish with the residue was allowed to cool in a desiccator, and was then carefully weighed, using a chemical balance sensitive to a milligram. It was found that the weighing process had to be carried out rather quickly since the deposit was very hygroscopic and rapidly absorbed moisture from the air, an exposure of a minute or so to the air being sufficient to alter its weight appreciably.

² It is convenient to term one part of dissolved matter in 100,000 parts of water a concentration of one "degree." The term thus falls in with the chemist's "degree of hardness."

still less from the rainfall data alone. In the case of the present pond the question at once arises, how is it that during certain months the concentration may be increasing when the water-level is rising? It has already been mentioned in Section II that during the autumn the very considerable quantity of floating leaves of *Potamogeton natans* are in process of decay, and there seems no doubt that the products of this decay, which must be considerable and which of course are returned to the water, are responsible for the marked increase in dissolved matter noted during October—November 1920, and September—November 1918 and 1919. Moreover, as will be referred to later in Section V, during the autumn months (September—November) very considerable masses of *Edogonium*, growing between the leaves of the *Potamogeton natans*, gradually decay, and the resulting products must also have contributed to the increase in dissolved matter noted during the autumn months.

Later on (in Section V) reference will be made to a great maximum of *Zygnema*, culminating in May (and occurring in all four years), but a quick decline setting in during June, at the end of which month this Alga is generally rare. Now between May 8th and May 29th, 1920, the water-level fell 2.5 cm., while the concentration at the same time increased from 9 to 18 degrees—an amount which it seems impossible to account for by the mere evaporation of the water; and from May 29th to June 17th of the same year the water-level rose 1.3 cm., but notwithstanding this dilution the concentration increased still further to 20 degrees. There is some evidence, therefore, that the products of the decay of *Zygnema* are partly responsible for this increase in dissolved matter observed during May and June, 1920. During these months in 1919 the water-level was falling, so that it is not possible to say how far the rising curve of dissolved substances was the result of decay of Algae, or of mere evaporation.

It will be convenient here to refer to some periodic determinations of the freezing-point of pond-water made by Transeau (28) during 1913–1915. He states that his results “indicate that the highest concentrations coincide with the periods of greatest rainfall and higher water-levels, and the periods of low concentration are coincident with low water-levels and drought” (*l.c.* p. 131). He considers that these results are due to the rains bringing in soluble salts from the upper layers of the soil, and by the silt, clay and suspensoids, also brought in by the rains, slowly settling to the bottom of the pond and carrying “nearly all the soluble salts with them.” He limits this statement to “pools, ponds and streams fed by surface run-off,”

and mentions that the underground water of a well gave opposite results.

These conclusions certainly do not apply to Hawkesley Hall Pond (which nevertheless receives most if not all of its water from surface-drainage of the land), as even a cursory examination of the curves of Fig. 3 will show; and even in the cases noted where the concentration of the water increases simultaneously with a rise of the water-level, the explanation put forward by Transeau in the case of the American ponds is not adequate to explain the facts in the present instance. Of course it must be borne in mind that the amount of soluble matter carried into a pond by drainage-water depends very largely upon the geological nature of the rock-strata in the neighbourhood of the pond; and this fact alone would very seriously interfere with the general applicability of the results obtained from Transeau's examination of ponds in central Illinois.

From what has been said already there is reason to believe that the water of Hawkesley Hall Pond contains rather large amounts of organic matter in the form of soluble peaty compounds resulting from the decay of the various phanerogamic and algal species, and a few determinations were carried out to ascertain roughly the proportion of these organic substances to the inorganic salts in the water.

With regard to the hardness of the water of the pond, which was determined¹ each month from June 1920 onwards, the following table gives the results obtained:

Table showing the Degrees of Hardness² of the Water of Hawkesley Hall Pond from June 1920 to June 1921.

		1920							1921					
		May 29	June 17	July 15	Aug. 7	Sept. 18	Oct. 16	Nov. 13	Jan. 8	Feb. 12	March 12	April 16	May 14	June 11
Temporary Hardness	}	3·5	3·3	3·3	3·8	3·9	3·9	3·8	3·6	3·4	3·5	3·6	4·0	4·5
		0·5	0·5	0·5	0·6	0·6	0·6	0·6	0·5	0·5	0·5	0·5	0·6	0·6
Total Hardness		4·0	3·8	3·8	4·4	4·5	4·5	4·4	4·1	3·9	4·0	4·1	4·6	5·1

From this table it is obvious that the hardness of the water between May 1920 and June 1921 tended to remain remarkably constant, so

¹ By Hehner's method, as described, for instance, in Newth's *Manual of Chemical Analysis*, p. 328.

² Temporary hardness is due to calcium or magnesium carbonate, permanent hardness to (mainly) calcium or magnesium sulphate. A degree of hardness is equivalent to 1 part of CaCO_3 in 100,000 parts of water.

that the rather wide fluctuations (between 12 and 20 degrees) in the total amount of dissolved matter of the water (Fig. 3) during these dates is very probably due for the most part to variations in the quantity of dissolved organic matter, and not of the inorganic salts.

A single experiment was made to determine roughly the amount of organic matter in the water by the method of ignition¹. On September 18th, 1920, a known weight of the deposit, obtained by evaporation over a steam-bath in the ordinary way, was heated to redness on a piece of platinum foil, and the loss in weight ascertained by weighing. The original weight of the deposit was 0.026 gram, the residue after ignition weighed 0.010 gram, the loss in weight (representing chiefly organic matter) thus being 0.016 gram; or, in percentages, roughly 61.5 per cent. organic matter and 38.5 per cent. inorganic salts. On the same date the total amount of dissolved matter was 13 degrees (= 0.013 per cent.), and from the above experiment we see that roughly 8 degrees of this represents dissolved organic substances, and 5 degrees inorganic salts. Now since on this date the total hardness of the water (by the acidimetric method) was 4.5 degrees there remains only 0.5 degree to be reckoned as inorganic salts other than those responsible for the "hardness" of the water.

Thus it is probably correct to say that only 0.2 to 0.4 of the deposit, as obtained each month by evaporation over the water-bath, was composed of inorganic salts, the rest being organic matter; and that while the inorganic part of the deposit varied from month to month apparently between comparatively narrow limits, the amount of organic matter fluctuated between much wider limits; so that the marked variation observed in the total dissolved matter of the water during the period of observation is mainly to be accounted for by this rise and fall in the amount of soluble organic matter.

V. THE ALGAL FLORA OF HAWKESLEY HALL POND

The pond is very rich in species of Algæ, and the list has been divided into three groups: (1) those Algæ which at certain times have formed a really conspicuous feature of the algal vegetation of the pond; (2) those which have developed at times only to a moderate extent, or have been only locally common at times; and (3) those which have been rare or of isolated occurrence.

GROUP 1 includes: *Cryptomonas ovata*, *Trachelomonas volvocina*, *Chlamydomonas Reinhardi*, *Eudorina elegans*, *Pandorina Morum*,

¹ This method, of course, is not very accurate for the determination of organic matter in water, but it will serve to give a rough idea.

Dictyosphaerium Ehrenbergianum, *Tribonema bombycinum* and its forma minor, *Ædogonium cryptoporum*, *Æ. crispum*, and other species in the vegetative condition, *Spirogyra calospora* (= *protecta*), *S. inflata*, *S. colligata* (20), *Zygnema* sp., *Hyalotheca dissiliens*, *Cosmarium Botrytis*, *Staurastrum brevispinum*, *S. Dickiei*, *Xanthidium antilopæum*, *Closterium striolatum*, *C. moniliferum*, *Anabæna oscillarioides*.

GROUP 2 contains: *Trachelomonas hispida*, *Synura uvella*, *Volvox aureus*, *Scenedesmus denticulatus* var. *linearis*, *Ankistrodesmus falcatus*, and its vars. *acicularis*, and *spirilliformis*, *Pediastrum Boryanum*, *P. tetras*, *Cælastrum sphaericum*, *Chætophora pisiformis*, *Draparnaldia glomerata*, *Aphanochæte repens*, *Tribonema affine*, *Vaucheria aversa*, *Ædogonium Borisianum*, *Æ. Braunii*, *Æ. echinospermum*, *Mougeotia scalaris*, *M. viridis*, *Spirogyra catenæformis*, *S. longata*, *S. nitida*, *Sphærozozma granulatum*, *Pleurotænium Ehrenbergii*, *Staurastrum furcigerum*, *Closterium Dianæ*, *C. acerosum*, *C. Kützingii*, *C. lineatum*, *Oscillatoria tenuis*, *O. splendida*, *Phormidium uncinatum*, *P. molle*, *Cylindrospermum majus*.

GROUP 3 includes: *Euglena viridis*, *E. sanguinea*, *E. spirogyra*, *E. tripteris*, *Phacus pleuronectes*, *P. longicauda*, *P. pyrum*, *Trachelomonas oblonga*, *Chlamydomonas Debaryana*, *C. monadina*, *Gonium sociale*, *G. pectorale*, *Tetraëdron minimum*, *Oocystis solitaria*, *Glæocystis gigas*, *Characium longipes*, *Apiocystis Braunii*, *Microspora floccosa*, *M. stagnorum*, *Coleochæte scutata*, *C. Nitellarum*, *Vaucheria sessilis*, *Ædogonium rugulosum*, *Bulbochæte minor*, *B. intermedia*, *Spirogyra condensata*, *S. varians*, *S. Jurgensii*, *S. mirabilis*, *S. Weberi*, *S. maxima*, *S. bellis*, *S. Grevilleana*, *Cosmarium reniforme*, *C. impressulum*, *C. præmorsum*, *C. Regnellii*, *Staurastrum inflexum*, *Penium Navicula*, *Arthrodesmus convergens*, *Closterium aciculare*, *Cl. Leibleinii*, *Cl. Ehrenbergii*, *Cl. rostratum*, *Cl. Venus*, *Gonatozygon Kinahani*, *G. monotænium*, *Arthrospira Jenneri*, *Tolypothrix lanata*, *Merismopedia elegans*, *Amphipectra pellucida*, *Synedra Ulna*, *S. radians*, *Fragillaria capucina*, *F. mutabilis*, *Tabellaria fenestrata*, *Gomphonema parvulum*, *G. acuminatum*, *Epithemia turgida*, *Nitzschia Palea*, *N. acicularis*, *Navicula viridis*, *N. vulpina*, *N. major*, *N. mesolepta*, *N. cryptocephala*, *Stauroneis anceps*, *S. Phænicenteron*, *Cocconema gastroides*, *C. Cistula*, *Amphoru ovalis*, *Cyclotella Meneghiniana*.

VI. GENERAL ACCOUNT OF THE ANNUAL CYCLE OF THE PREDOMINANT ALGAL SPECIES

It has been found convenient to consider the whole algal vegetation of the pond as inhabiting two more or less distinct provinces,

these being (1) the central region of the pond, corresponding roughly with the central area of *Potamogeton natans* (see Section II), and (2) the peripheral region of shallow water. The second region is more especially developed on the east side of the pond (cf. Fig. 1) where the bottom slopes very gradually, producing a rather wide marshy zone of shallow water, which at times is particularly favourable for the development of many algal species.

The Algæ of these two provinces are for the most part remarkably distinct throughout the greater part of the year, although of course there is no marked line of separation between the two regions, and many species are found in both, although the dominant Algæ in the two provinces are generally different. It is not considered that the algal vegetation of all small ponds exhibits this distinction; it only happens to obtain in the present case.

Two communities may thus be distinguished in the algal vegetation, namely a Central Community inhabiting the deeper water, and a Shallow-water Community¹ near the sides of the pond. In the Central Province of the deeper water four phases may be distinguished in the annual cycle, these phases corresponding roughly with the four seasons, although the spring- and summer-phases are the most marked. These four phases are:

(a) a Spring-phase (February to May) with *Zygnema* the dominant species;

(b) a Summer-phase (June to August) with *Edogonium* dominant, and *Anabæna oscillarioides* common (but *Zygnema* rare);

(c) an Autumn-phase (September to November) marked by a decided decrease in *Edogonium* and a slight increase in *Zygnema*; and

(d) a Winter-phase (December to January), transitional in character, with filamentous Algæ generally scarce, but *Zygnema* usually commoner than *Edogonium*.

The *Zygnema* (see Section VIII) usually increases rapidly during February, and by May has attained a great maximum, masses of the Alga occupying nearly all the free water-surface between the floating leaves of *Potamogeton natans*. During the spring, however, a wide species of *Edogonium* (see Section X) gradually increases in amount and by June replaces the *Zygnema*, which rapidly declines during this month. From June to August *Edogonium* is at a maximum, and is often even more abundant than was the *Zygnema* in spring; while *Anabæna* is usually common amongst the *Edogonium*

¹ At certain times of the year several more or less distinct communities or facies may be distinguished in this shallow-water region.

during July and August. In the autumn *Edogonium* gradually decreases in amount, and by November has generally become rather scarce; but the *Zygnema* again develops during September and October, and in general shows a steady increase up to the following spring maximum.

In the Shallow-water Province, however, rather more species are concerned in the annual cycle. Roughly three phases can be distinguished each year, and of these the spring-phase coincides (as regards the season) with the spring-phase observed in the Central Province. The three phases are:

(a) a Spring-phase (February to May) with *Spirogyra* spp. predominating, and *Tribonema bombycinum* gradually decreasing, while *Mougeotia scalaris* may be locally common during May;

(b) a Summer-phase (June to September) with *Anabæna*¹ dominant, and *Cylindrospermum* locally common; and

(c) a late Autumn and Winter-phase (October to January) with *Tribonema bombycinum* the chief species, while *Spirogyra* spp. may be locally common, and *Microspora* more or less developed during the winter.

There is much more variation in the annual cycle shown by the Algæ of the shallow marginal region than in that observed in the Central Province of the pond, and this variation can in many cases be explained by a consideration of the various factors (temperature, concentration of the water, bright sunshine, etc.) which control to a large extent the growth of freshwater Algæ in nature. This matter will be discussed in the following sections.

Perhaps the most obvious point of distinction between the general annual cycle of the algal vegetation of Hawkesley Hall Pond and that described by Fritsch and Rich, both in the case of Abbot's Pool (17) and also Barton's Pond (18), is the absence of any distinct Diatom-phase during the colder months in the pond now under consideration. Diatoms never played more than a mere subsidiary part in Hawkesley Hall Pond, although a fair number of species were observed in it.

The more detailed discussion of the periodicity of the chief algal forms represented in the pond now comes under consideration.

¹ *Anabæna* also extends into the deeper water during the summer months.

(To be continued.)

THALASSIOPHYTA AND THE ALGAL ANCESTRY OF THE HIGHER PLANTS¹

By F. E. FRITSCH

THE publication by Church in 1919 of his "Thalassiphyta and the Subaerial Transmigration²" introduces in the main two new aspects in connection with the origin of land-plants. The first of these substitutes for the old view of invasion of the land *via* rivers and other pieces of freshwater, a conception of direct evolution from Seaweeds gradually becoming adapted to terrestrial conditions as they were left exposed on rising sea-bottom. The second hypothecates that the first land-plants originated, not from simple filamentous Algae, but from bulky Seaweeds of some considerable dimensions, exhibiting oogamy and fertilisation *in situ* and possessed of a regular alternation between an asexual diploid and a sexual haploid phase. Church's fascinating memoir deals at great length with these two theories and all that they involve, and numerous facts are adduced in their support. At the same time it seems to me that not all the available ground has been explored and that, particularly as regards the second concept, there are certain facts which indicate the possibility of a different conclusion.

It is generally agreed that the precursors of terrestrial plants must have been green, starch-producing Algae (cf. Church, *loc. cit.* p. 88), since other lines of Seaweeds possess a photosynthetic mechanism which does not appear to have been successful on land. Green Algae (apart from the highly specialised line of the Siphonales), however, play a relatively subordinate part in the sea, both as regards number of species and individuals. Moreover no Green Alga has attained to any really considerable dimensions or bulky parenchymatous construction, and it is significant that in this respect there is nothing to choose between freshwater and marine Cladophoraceae or Ulvaceae. In fact it may be said that Chlorophyceae as a whole possess no somatic organisation such as has been evolved

¹ From the Botanical Department, East London College, University of London.

² *Oxford Botanical Memoirs*, No. 3.

by the Phaeophyceae and Florideae. The same statements apply to the Cyanophyceae and, except as regards relative abundance, also to the Diatoms¹. In short all those phyla which have been really successful in freshwater and terrestrial habitats exhibit no greater development in the sea than on land and are in fact, except in the case of Diatoms, relatively poorly represented in the former. Even the Siphonales, which exhibit a comparatively high vegetative development, are, in their reproductive features, on a lower plane of development than many Brown and all Red forms and in this respect are at a lower level than the freshwater representatives. These, *Vaucheria* and *Dichotomosiphon*, are the only oogamous members of the series². Such forms as *Trentepohlia*, *Draparnaldia*, and *Coleochaete*, in one feature or another, show indications of higher equipment than is found in any marine Green Alga.

In view of the dominance of Chlorophyceae and Cyanophyceae in freshwater and terrestrial habitats it appears warranted to conclude that for them conditions of life in such situations have been more favourable than in the sea, where perhaps they have been unable to hold their own against the more successful Brown and Red forms. These latter do not appear to take kindly to freshwater, and such few examples as are known (*Hildenbrandtia*, *Lemanea*, *Batrachospermum*, *Lithoderma*, etc.) are largely characteristic of rapid streams, many of the Red forms betraying their extreme light-sensitiveness by frequenting the underside of overhanging rocks and other shaded habitats. There is considerable probability in these cases of direct estuarine migration (Church, *loc. cit.* p. 8).

Church holds the view that "the Green Algae surviving in freshwater are somatically the merest depauperated relics in the last phases of deterioration, as reduced filamentous and disc-types" (p. 8). Presumably he would apply the same explanation to the Blue-green Algae, for on p. 32 he speaks of "the flora of freshwater ponds and freshwater Algae, presenting types of organism so far removed from their original condition." That series of reduction of filamentous forms can be recognised in some of the lines of Chlorophyceae is unquestionable, but there is no evidence of reduction in the group as a whole, that is to say there is nothing to show reduction from forms organised on a higher somatic basis. The groups Chloro-

¹ Church (*loc. cit.* p. 47) cites the marine *Schizonemas* and *Licmophora* as instances of higher organisation amongst marine Diatoms, but the difference as compared with some of the freshwater colonial forms is not very great.

² *Sphaeroplea* might be added, but it is doubtful whether it belongs to this series.

phyceae and Cyanophyceae can equally well be regarded as having remained at a low horizon of development.

It may moreover be pertinently asked in this connection why, if freshwater Algae are reduced, the marine Chlorophyceae (and Cyanophyceae)—in what Church considers to be the more favourable environment—do not exhibit any higher organisation than their freshwater allies. Church (p. 30) contrasts the freshwater *Vaucheria* with the marine *Codium*, the former being regarded as having a reduced thallus, but, apart from the absence of all evidence of reduction, there are a considerable number of marine Codiaceae (*Aurainvillea*, *Udotea minima*) in which the organisation of the thallus is not much in advance of a *Vaucheria* or *Dichotomosiphon*¹. As far as I am aware too, there is nothing to choose between the marine and freshwater Phanerogams, either as regards dimensions or prolific growth. In short a comparison of the existing forms which grow in both types of habitats far from supporting the view advanced by Church seems to indicate that conditions of plant-growth in the sea are less favourable for green plants than in freshwater.

Church points out (p. 7) that the chemical content of fresh as opposed to salt water is strikingly inferior, a fact about which there can be no difference of opinion, but this does not necessarily imply that nutrition is or was easier in salt than in freshwater. Too little is known about the nutrition of Seaweeds to enable one to express an opinion on the point². Moreover it may be doubted if we can safely assume that the primeval sea was at all as rich in mineral salts as that at the present day; according to Schuchert³ "the Archeozoic oceans had far less salts and probably a different salt combination." The change from marine to freshwater conditions in those remote times may not have been anything like as pronounced as one as it would be at the present day and this may perhaps be the clue to the reason why transmigration has not occurred in more recent epochs, modern Seaweeds being too highly adapted to their

¹ Church (cf. *loc. cit.* pp. 34, 46) accepts West's view that the Desmids, certainly among the more successful and ubiquitous Green Algae, are reduced from filamentous forms. It has however repeatedly been pointed out (Tansley, in *New Phytol.* 4, 1905, p. 145; Fritsch, in *New Phytol.* 16, 1919, p. 5) that all the evidence cited in favour of this view will read equally well the other way. Few algologists would follow Church (p. 8) in regarding *Hydrurus* as a reduced filamentous type.

² On the whole Green Algae are more accommodating as regards considerable variations in the concentration of the surrounding medium than the true Seaweeds (cf. for instance Oltmanns' *Morph. u. Biol. d. Algen*, 2, 1905, p. 178).

³ *New Phytol.* 19, 1920, p. 274.

concentrated environment to withstand so radical a change (cf. also p. 178).

Much of the evidence cited by Church as illustrating malnutrition in freshwater is open to a different interpretation. The relevant features of estuarine and salt-marsh vegetation, which he (p. 7) attributes to withdrawal from the open sea, may equally well be a result of the frequent changes in concentration of the medium owing to tidal influence. The statement that "all freshwater Plankton is on a lower plane in size, variety, and abundance" (footnote 4 on p. 7) is also open to question. There is no doubt a difference as regards size, but is that not more plainly related to the greater buoyant power of sea-water? As regards variety, however, it may be doubted if there is any appreciable difference, and under certain circumstances, as in warm waters (especially Tropics¹), the abundance of freshwater Plankton may quite well equal that customary in the sea.

It does not appear therefore that there are any adequate grounds for the views (a) that conditions in freshwater are necessarily less favourable to growth than those in salt water, or (b) that freshwater Algae as a whole are reduced forms. It is however a striking fact that all Green Algae (excl. Siphonales) leave off abruptly at a level of morphological development which is far inferior to that exhibited by the true Seaweeds. It is at least a plausible assumption that Green Algae do not pass beyond this level, because all the more advanced forms have progressed landwards and given rise to the higher land-plants. They alone (likewise the Cyanophyceae) seem to have found more suitable conditions on the land and in freshwater than in the sea. The conclusion is that the transmigrant Green Alga was at about the level or perhaps slightly above the level of the present-day forms, at least in morphological development. The absence of more advanced forms in the series of the Green Algae is, on this basis, explained as due to failure to compete successfully in the sea with the Brown and Red forms and, for the rest, to their evolution into land-plants.

It is particularly to be noticed that Green Algae (as also Cyanophyceae and to a lesser extent Diatoms) show a very marked power of adaptation to life on land. In practically every series of Green Algae, as at present distinguished, there are terrestrial representatives; as illustrated by the following epitome:

Tetrasporales: species of *Palmella* and *Gloeocystis*.

¹ Cf. Fritsch, *Proc. Roy. Soc. London*. Ser. B. 79, 1907, p. 220.

Protococcales: numerous forms (species of *Chlorococcum*, *Chlorella*, *Trochiscia*, etc.).

Ulotrichales: species of *Hormidium*, *Prasiola*, *Stichococcus*, *Microspora*, *Rhizoclonium*.

Chaetophorales: *Trentepohlia* and other members of *Trentepohliaceae*; *Pleurococcus* (?).

Oedogoniales: *Oedocladium*.

Siphonales: *Vaucheria*.

Conjugatae: species of *Mesotaenium* and *Cylindrocystis*; *Zygogonium ericetorum*. (See also Bristol, *Ann. of Bot.* **34**, 1920, p. 35.)

The extent to which such terrestrial Algae are developed is probably not generally familiar. Everyone is of course acquainted with the ubiquity and abundance of *Pleurococcus*, but *Zygogonium ericetorum*, *Hormidium flaccidum*, species of *Prasiola*, and species of *Trentepohlia* are scarcely less common and the first-named at least often covers whole acres of ground¹. On the other hand I know of no records of indubitable Brown or Red Algae as inhabiting equivalent terrestrial situations. Where conditions are less favourable to the growth of Green Algae it is *Cyanophyceae* that dominate as terrestrial algal forms².

A large number of these terrestrial *Chlorophyceae* and *Cyanophyceae* possess a faculty for resisting drought, without any assumption of special resting-stages, that is only paralleled elsewhere among Lichens and Mosses³. It is certainly not the bulky parenchymatous forms at the present day that are best equipped to resist desiccation. If Church cites *Pelvetia* and the *Fuci* of salt-marshes as instances of bulky forms able to maintain themselves in semi-aquatic environment (p. 21), the numerous terrestrial *Trentepohlias* and other terrestrial forms, such as *Prasiola*, *Hormidium*, and *Zygogonium*, show the great capacity of filamentous Green Algae to maintain themselves, *even in a dry atmosphere*. The terrestrial Green Alga is indeed highly adapted to its conditions of life and it may have been this faculty, apparently possessed otherwise only by the Blue-green Algae and some Diatoms, that primarily resulted in the success of Green forms in establishing themselves on dry land.

A leading hypothesis in Church's theory may be summarised in his own words (p. 33): "The whole of the fundamental framework

¹ Recent Antarctic exploration has shown that *Prasiola* is probably the most important green plant in the land-vegetation of these latitudes.

² Fritsch, *loc. cit.* p. 203 et seq.

³ Cf. West and Starkey, in *New Phytol.* **14**, 1915, p. 201; Piercy, in *Annals of Botany*, **31**, 1917, p. 527 et seq.; Fritsch, in *Annals of Botany* (in the press).

of the organisation of a land-plant, the anatomy of its tissues, the morphological differentiation of members, as also the otherwise wholly inexplicable sequence of sexual and asexual phases in the life-cycle, are the expression of response to the conditions of marine environment." It is difficult to conceive of any factor or group of factors in marine environment that could be interpreted as especially stimulating evolution in these directions. Is it not more likely that the equipment evolved alike in the sea and in the land-flora is an expression of the general trend of evolution in vegetable organisms? Among Seaweeds (Siphonales, Ectocarpales, Florideae) a massive soma has been evolved from the primitive filamentous condition by several different methods, and are we to suppose that marine environment alone stimulates such evolution and that an analogous origin from simple filamentous forms would be impossible on land? As regards morphological differentiation of members, a flattening of certain portions of the shoot-system for purposes of photo-synthesis is surely a response which can be postulated as readily under terrestrial as marine conditions, and is indeed observed in many "xerophytes" as cladodes and phyllodes, undoubtedly evolved on the land; such flattening is moreover evident in the terrestrial *Prasiolae*. Development of branches (axillary only in Phanerogams and a few others), in close connection with such foliar expanses, is probably a necessary physiological consequence of the localised assimilatory activity. A somatic main axis once established, its downward growth into the soil as a root is not difficult to conceive without the necessity of supposing a derivation from the "crampons" of present-day Seaweeds. If *Rhynia* and *Psilophyton* are to be regarded as primitive Pteridophyta, it is possible that in some of the transmigrant races at least the establishment of roots and leaves was deferred to a fairly late stage.

An alternation between sexual and asexual phases must have come about as soon as the reduction-division became associated with spore-formation, for when the spores were haploid the plant to which they gave rise (gametophyte) was cytologically different from the other (sporophyte). Need we assume that such relegation of the reduction-division to the time of spore-formation could only occur in the sea? There does not appear to be any logical reason for such an assumption, since we know of nothing in marine environment that would specially favour such a direction of evolution. The fact that it exists in many Brown and Red Algae may be taken as merely showing that it is an inevitable trend of evolution in all advancing

plant-life. Why the plant, and not the animal, kingdom has adopted this scheme remains unanswerable (cf. also Fucales).

Among the Seaweeds we meet with great diversity in the character of the alternation, a fact which is best exemplified in the Phaeophyceae (cf. *Dictyota* and *Cutleria* for instance). When Church remarks (p. 25) "In all cases of heterothallic differentiation, the individual derived directly from the zygote is the one which begins to diverge from the type, and expresses deterioration," he overlooks Sauvageau's important work on alternation of the Laminariales¹. Moreover neither the Ectocarpales nor the simple filamentous Green Algae of the sea show this phenomenon. Even among the Red Algae the Nemalionales exhibit a simple type of "antithetic" alternation which is not much removed from that of *Coleochaete* among Green forms, except perhaps in the incidence of the reduction-division. The fact that there may be no alternation in a considerable bulk of Seaweeds and that there is great diversity in the character of such alternation appears to lend support to the view that alternation need not necessarily only have evolved under marine conditions, but has been a phase of evolution of the Vegetable Kingdom.

Although *Coleochaete* is the only green form that exhibits any definite type of alternation, the potentialities for such an alternation are, as will be shown below, quite evidently present². The absence of definite alternation in most Green Algae (of sea and freshwater alike) may be taken to imply either (a) that alternation in green plants was evolved subsequent to the adoption of a land-life, or (b) that those Green Algae that had already acquired alternation became the successful transmigrants³.

In Church's memoir, where the primeval ocean is supposed to have existed before there was any dry land, the origin of a terrestrial flora is postulated as occurring in connection with the gradual elevation of vast continental stretches of sea-bottom above the water-level. What evidence have we that when such elevation took place the Benthos had attained to anything like the stage of development that modern Seaweeds exhibit? Church has sketched for us the fascinating picture of the evolution of Benthic from Planktonic forms as the ocean-floor was raised and the sea became locally

¹ Cf. for instance, *Comptes Rendus de l'Acad. d. Sci.* t. 161, 1915.

² Cf. also Fritsch, "The algal ancestry of the higher plants," *New Phytol.* 15, 1916, p. 233 et seq.

³ It is however not easy to suppose that in such a case no form exhibiting alternation should have been left behind.

shallower. Assuming the rise to have been more or less continuous, however gradual, would the period of time elapsing between the establishment of a Benthos and the first emergence of land above the surface be anything like sufficient to suppose that the Benthic Seaweeds could have attained to the high stage of development that Church postulates? It seems improbable.

Moreover, according to the geological testimony¹ there was no continuous ocean *ab initio*; there always was land and apparently more of it than at the present day. That being so, there were probably, as in later geological epochs, frequent earth-movements leading, on the one hand, to subsidence of land and, on the other, to elevation of previously submerged sea-bottom. There may thus have been, always accepting Church's theory as to the method of establishment of the land-flora, repeated transmigrations, some of which occurred at a much earlier epoch than others. It may, for example, be suggested that the Blue-green Algae, with their imperfect cytological differentiation, lack of a sexual process, and very simple morphological construction, were transmigrants at an epoch when the evolution of algal life had not passed beyond this horizon. Their world-wide distribution is quite in accord with such a view. The Green Algae, already at a higher stage of development (complete cytological differentiation, sexuality, higher morphological construction), may have adopted terrestrial existence at a much later period, leaving only the siphonous series to develop vigorously, mainly in the warmer seas. It may be freely granted that there are also possibilities of transmigrations at still later periods, the transmigrants being highly organised Seaweeds as Church supposes. The considerations put forward in the preceding pages do not however appear to lend countenance to this view which moreover necessitates the assumption that at that far distant epoch evolution in Seaweeds had practically completed itself² and that there has been no appreciable change since. It may be questioned too whether such highly developed forms, as Church conceives the transmigrants to have been, would adapt themselves as readily to the conditions of a land-life as simpler Algae would.

The changes involved in the assumption of a land-life are very fully considered by Church and, as regards the probable conditions

¹ Schuchert, *loc. cit.* p. 272.

² This is the view actually taken by Church, cf. p. 10. His reference to the *Solenopora* of the Lower Carboniferous is scarcely relevant in this connection, since at that time the land-flora was already highly developed and we know little of the reproductive mechanism of these forms.

to which the transmigrants were subjected, there can be little difference of opinion except as regards illumination and the moisture-content of the air. On these points Church himself is not clear, since on p. 20 he speaks of "a subsaturated atmosphere and a sky of fog and cloud" and on p. 21 of "the brilliance of open sunlight." It is difficult to conceive of any possibility of transmigration, whether by filamentous or more massive forms, unless the air was almost saturated and there was copious rainfall during the greater part of the period. Even if that was so, however, there must have been an appreciable increase in light-intensity for an organism emerging from the water, and it is difficult to follow Church when he suggests (p. 34) that "types with the elaborated factors of such shade-flora (Florideae) will have the better chance of survival under conditions of malnutrition." All recent experimental work goes to show that shade-loving Algae are extremely sensitive to any increase in light intensity.

The most serious problems for the transmigrant must have been water-supply and the maintenance of an erect habit, since it may be doubted (cf. above) whether the change in the nutritive value of the solution was at all as profound as Church supposes¹. The abundant terrestrial Algae belonging to the successful groups testify to the capacity of these to exist through a period of diminished water-supply and, as above suggested, this capacity may have been one factor that led to their success in transmigration. It may be granted that it is not easy to picture the origin of even the simplest land-plant from a filamentous Alga, but there is no more difficulty in it than in accepting Church's view. The larger the transmigrant the more acute would be the problem of water-supply at first and it is difficult to conceive of the persistence of an erect Alga 3-6 feet long (Church, p. 90), gradually raised out of the water; and if the transmigrant was not erect from the first, it is not easy to comprehend how such a habit could be subsequently attained².

It may be that the land- and water-forms of present-day aquatics give us some indication of what the effect of the assumption of the terrestrial mode of life may have been, viz. a general condensation

¹ As regards this point, if freshwater Algae are derived from marine forms and have been able to survive the "malnutrition" involved in the change, why should not similar forms have survived and gradually developed into higher land-plants?

² Church (p. 21) cites an observation of Migula's on *Nilella mucronata* growing erect to a height of 10 inches in subsaturated air "with no other mechanical assistance than the turgidity of its non-corticated axial cells," but since such a plant has little weight to support it can scarcely be regarded as affording evidence for his hypothesis.

and dwarfing of parts whereby the exposure of any large surface or the elevation of any part of the thallus into the drier air some little way above the surface was obviated. Such dwarfing may be seen in the spray zone of larger pieces of water at the present day, in the case of *Cladophora*, *Oedogonium*, *Stigeoclonium*, etc. The gradual origin of an erect growth from such short tufted filaments, as they became accustomed to terrestrial conditions, is not as difficult to picture as the persistence of a large erect form.

It is, however, possible that an erect growth was not the most important feature of the transmigrant Alga. In each of the three great series of Algae we encounter a similar central type of organisation, in which the thallus is differentiated into two distinct portions, a prostrate attached dorsiventral base, which not uncommonly possesses a parenchymatous construction, and an "erect" radial portion. This is well exemplified in the species of *Stigeoclonium* and *Trentepohlia* among the Chaetophorales (Chlorophyceae), in *Ectocarpus*, *Cutleria*, etc. among the Phaeophyceae, and in several of the Nemalionales among Florideae. In the Seaweeds such an organisation appears on the whole to be most marked among the simplest members of their respective series, whilst the Chaetophorales, the bulk of which are freshwater or terrestrial, are among the most advanced members of the Green Algae¹. It seems probable that this type of construction marks a stage in the evolution of the Algae, the highest attained by the Green forms of the present day.

In each of the series just mentioned we find, side by side with forms with a practically equal development of the prostrate and "erect" portions, others in which the one or the other is more or less completely reduced (among Chaetophorales compare *Draparnaldia* and *Protoderma*). In the aquatic Green forms the reproductive organs are usually borne on the upright system, but in some of the terrestrial *Trentepohlias* the spherical sporangia, which have been shown in several cases to be gametangia, are confined to the creeping base², whilst the asexual stalked sporangia, so highly evolved for aerial dispersal, are found on the "erect" system. Among Phaeophyceae, in *Myrionema vulgare* and *Cutleria* (in the latter on two distinct generations), the converse is the case. I have previously suggested³ that the Algae from which land-plants arose possessed

¹ Fritsch, *loc. cit.* p. 235.

² Cf. Heering, in Pascher, *Suesswasserfl.* 6, 1914, Fig. 173, a; De Wildeman, *Algues Buitenzorg*, 1900, p. 72; Schmidle, in Engler's *Bot. Jahrb.* 30, 1902, p. 63, Tab. II, Figs. 8, 10.

³ *Loc. cit.* p. 240.

such a thallus developed in two directions (as indeed present-day *Trentepohlias* illustrate) and that as an adaptation to subaerial conditions relegation of the sexual reproductive organs to the creeping base took place. Such a theory is in harmony with the prevalent dorsiventrality of the gametophyte and helps to explain the persistence of "aquatic" fertilisation in Bryophyta and Pteridophyta¹. Whether alternation was already established or whether it became subsequently established is immaterial; as soon as two phases were evolved they would be bound gradually to diverge, the one (gametophyte) losing the erect system (except in Mosses?), the other (sporophyte) the prostrate system. In the dual development of the primitive type of thallus we have all the potentialities for the evolution of the two highly contrasting phases in the life-cycle of land-plants. On this view they were probably *ab initio* identical.

Let us consider the position of such a filamentous form, as it gradually became exposed by the rising of the land out of the water. Assuming the power of adaptation to terrestrial existence so strongly evidenced by the Green Algae of the present day and the general condensation of the exposed parts above postulated, we should have a dense tufted erect growth (probably of no appreciable height) arising from a creeping basal system which would probably not be as much affected at first at least². Such a form would not be subjected to the vicissitudes to which larger forms would be exposed and, with the more favourable conditions for photo-synthesis and the better oxygen-supply, might well make relatively rapid headway. It is difficult to conjecture what direction the advance might take at first, but there may well have been an early departure from the filamentous condition and the adoption of a more massive habit, either in both parts of the thallus or only in the creeping base. It may be an open question whether the transmigrant Alga had already attained to oogamy. There are no oogamous Green Algae in the sea (cf. Oltmanns, II. p. 176), so that oogamy may either have arisen subsequent to transmigration or the oogamous forms were the successful transmigrants³. At whatever stage oogamy may have been evolved fertilisation *in situ* and post-sexual nutrition would be a likely consequence of the increased efficiency of the organism due to the acquisition of a more massive body. It is to be noted that among Seaweeds the features in question are exemplified only in the

¹ Regarding Mosses, see Fritsch, p. 250.

² Since probably still periodically inundated.

³ In the latter case it is however again difficult to conceive that no oogamous forms should have been left behind.

Red Algae, a group so highly specialised that it may be doubted if it is warranted to draw any conclusions from them. On the other hand fertilisation *in situ* is a commonplace among freshwater Green Algae.

Church lays great stress on the fact that a resting thick-walled oospore is unknown among marine Algae, as little as it is found among land-plants above the level of the Thallophyta, whilst the oospores and zygospores of freshwater Algae invariably become equipped with a thick coat and pass through a more or less prolonged resting period. This is certainly a significant feature, but perhaps too much importance may be attached to it. We have to suppose that the asexual reproductive cells gradually acquired these features¹ as they became developed as wind-borne spores, and if they underwent the change why not the zygote? It is at least plausible that a resting stage was temporarily adopted by the zygospores or oospores during transmigration and that, as the thallus became more massive and post-sexual nutrition was initiated, this stage was again lost. A parallel is furnished by the evolution of the seed among higher land-plants, where the presence of a megaspore-membrane in Cycads, Conifers, etc. cannot but be taken as evidence of the former existence of thick-walled spores which, as nutritive devices developed, remained *in situ* and lost the thick membrane. Whilst therefore there is no reason to suppose that the zygotes of Seaweeds ever presented a resting stage (the conditions of life rendering it unnecessary), it is quite possible that such was realised during transmigration and retained as an advantageous device by freshwater Algae liable to desiccation, though subsequently lost in the main lines of landward evolution. Transference of the resting stage to the asexual reproductive units would present little difficulty since many of the simpler Algae (e.g. *Ulothrix*, *Stigeoclonium*) exhibit the power of initiating asexual resting cells side by side with the resting zygote. In fact the marked capacity for forming resting stages by several different methods (akinetes, aplanospores or encapsuled zoospores, zygotes) may have been another of the factors that led to the success of the green phylum.

A necessary adjunct to a successful terrestrial existence was no doubt the development of a more perfect type of sexual organ such as is exemplified by the archegonia and antheridia of present-day Bryophyta and Pteridophyta, and the preliminary steps in this

¹ In this connection attention may be drawn to the facility with which most freshwater Algae adopt a thick-walled resting condition on the part of structures that do not normally exhibit these features (akinetes, aplanospores, etc.).

direction may well have been taken at an early stage of transmigration. Church is of the opinion that "the archegonium is a derivative only of a truly parenchymatous type of soma" (p. 13), but it may be doubted if that is necessarily the case. *Ectocarpus* shows us how a multiseptate reproductive organ can arise in forms which are in the main monosiphonous and it may be questioned as to how far such a multiseptate organ is really removed from a gametangium of *Ulothrix* or similar form where there is likewise division along several planes, although the resulting units do not become separated by walls. It is not difficult to suppose the gradual evolution of archegonia and antheridia from a gametangium producing a considerable number of gametes, by the gradual modification of those of the peripheral series into a protective cellular wall. But the matter cannot pass beyond the realms of pure speculation and it is possible to visualise a number of different possibilities. There do not however appear to be any grounds for assuming that even a very early prototype of these organs had been evolved prior to transmigration, although that may have been the case.

Church regards the archegonium as being very probably polyphyletic, a view with which one is inclined to sympathise. The regular occurrence of a ventral canal cell seems to me, however, to place considerable difficulties in the way of its adoption and to imply a common ancestry for all archegoniate forms. Such a feature is difficult to explain on a theory of convergence. In supporting a polyphyletic origin for the different groups of land-plants (*e.g.* p. 82) Church places considerable weight on the type of flagellation of the spermatozoids. It may be doubted however if this feature always possesses the importance which is nowadays attributed to it, since several examples of the existence of diverse types of flagellation in one and the same form are known (*cf.* zoospores and spermatozoids of *Vaucheria* and the two forms of zoospores seen in some Saprolegniaceae). We may not lose sight of the possibility that the type of flagellation may in part be related to external factors of which we at present have little comprehension.

According to Church's theory (p. 89) "The Algae of the transmigration...combined the best features...of the known great conventional series of marine phytobenthon, and yet...belonged to none of them." In other words the Algae that gave rise to the transmitters have completely disappeared. That is plausible, but it is difficult to understand on this basis why Green Algae (as we find them at the present day) should almost alone have adopted life in

terrestrial and freshwater habitats and should be so poorly represented in the sea. These are facts which speak for a common origin of freshwater and terrestrial Green Algae and the higher land-plants and, since evidence of reduction in Green Algae as a whole cannot be admitted, it strikes at the root of Church's assumption.

At this point reference may be made to the other aspect of Church's theory, viz. the mode of transmigration. According to the present-day geological view there were both land- and sea-surfaces from the earliest times. If that was so, there was no doubt evaporation from the surface of the sea and subsequent atmospheric precipitation, which will have led to the formation of rivers and other large bodies of freshwater. We cannot preclude the possibility of an independent origin of green plant-life in such pieces of freshwater and of the ancestors of the higher plants never having been in the sea at all. Invasion of the sea by way of rivers on the part of a few freshwater Green Algae is not an impossibility and in the case of forms like Cladophoraceae and Ulvaceae even plausible in view of their present-day distribution. I hold no brief for either view, but consider that both possibilities must be reckoned with.

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THE STUDY OF HUMAN IMPLEMENTS AS AN AID TO THE APPRECIATION OF PRINCIPLES OF EVOLUTION AND CLASSIFICATION

By E. J. SALISBURY

EVERYONE who has attempted to teach the fundamental facts with respect to evolution and principles of classification has probably found the value of concrete examples in elucidating abstract concepts. But whether examples be culled from the animal or vegetable kingdoms there are always preconceptions in the student's mind with regard to them which tend to obscure the principles to be deduced from their study.

To overcome this difficulty the writer has resorted to the use of an analogy which the Editor has asked him to explain in the hope that others may find it equally helpful.

In the choice of such an analogy a wide field is offered us. If for example we wished to illustrate convergent evolution, probably no better examples could be found than the many isotopes which have now been demonstrated by the chemist and physicist possessing apparently identical chemical properties but different atomic weights. The two types of lead derived from uranium and thorium respectively offer as striking an analogy to the convergent evolution of the biologist as could well be found.

So too the morphologically identical Eucalypts, only separable by means of the terpenes which they contain, or the different albino *Primulas*, only distinguishable when analysed by the geneticist, are paralleled by the isomeric compounds of the organic chemist.

The periodic series into which the chemical elements fall and the light that has been shed by the study of radio-active substances have as profound a significance as the study of the passage from nebula to star and each serves to emphasise a particular aspect of the evolutionary sequence. Recently the former analogy has been developed by Heslop-Harrison in relation to his section-species of *Rosa* (*Trans. N.H.S. Northumberland, Durham and Newcastle*, vol. v, Pt. II, p. 257).

But though there is scarcely a branch of knowledge that cannot contribute something to the comprehension of evolution, especially in detail, the progress of development of man's implements appears

to offer a more valuable analogy with respect to general principles. We have here moreover the material for following the whole course of evolution from the primitive and crude efforts of prehistoric man through all the vicissitudes of increasing complexity and simplification up to modern times. The implement inherits its form by tradition from one generation to the next, whilst each age impresses its environment upon it.

Samuel Butler evidently regarded man's implements in a similar light but attached even more importance to them as the chief avenue of man's evolution (*Lucubratio Ebria*, 1865). To him the stage of man's organisation was measured by the number and variety of these extra-limbs at his disposal. Hence too the rich man was more highly organised than the poor man because of the variety of mechanisms at his command.

When the earliest stone implements are examined we are at once struck by the absence of clearly separable types. It is true these "eoliths" present various forms, but they bear comparatively little evidence of human workmanship and their differences depend rather on the character of the original stone selected than on its subsequent modification. There can be little doubt that these earliest implements served a variety of purposes, their very lack of differentiation rendering this possible. The eoliths are as it were the "Protista" of man's implements with many and various potentialities, some, though not all, of which we find realised in the increasing differentiation of succeeding epochs.

In the implements of the Strépy and Chellaeen periods, probably contemporary with Piltdown Man and the presence of the Hippopotamus and Rhinoceros in Europe, human handiwork is much more pronounced, though here again there is but little differentiation. So slight is this indeed that we should probably hesitate to regard them as distinct species were we dealing with biological entities. Here then we find emphasised the first fundamental principle, namely, that specific rank, which is after all an artificial aggregate of convenience, cannot have the same value in a group exhibiting little division of labour, as in one of highly organised units. There is more difference in the absolute sense between the multitudinous types of modern table knives than between the variations in the stone implements of the Chellaeen and Acheulean epochs, yet the distinctions of the former are unlikely to lead to any striking new advances in the evolution of the knife, still less to any entirely new implement. So far as we can judge they have no survival value.

If on the other hand we consider the heavy hand axe of the Chellaeen period, coarsely flaked on both faces, and compare it with the "ficron" type with its tapering point and more or less triangular section we realise as we pass to the Moustierian epoch that this type, though so little different from the normal one, was in reality the evolutionary starting-point of the pick-axe and the hand-drill, the origin that is of entirely new classes of implements.

A consideration of such examples helps one to realise that the differences in structure which serve to distinguish species of *Chlamydomonas* or *Bacteria* are necessarily small as compared with those separating *Capsella heteris* from *Capsella simplex* or *Silene nutans* from *Silene dubia*; yet such small differences in a relatively undifferentiated group, which has not lost its plasticity by division of labour, may be the inception of fundamental evolutionary changes. The value of the character, whether in the organism or organ, must be considered relative to the complexity or simplicity of its organisation.

When we pass from the Chellaeen to the Acheulaean period the diminishing size of the flaking involving a higher grade of technique seems a relatively small development, but this increased dexterity made possible the thinner implements of the Moustierian period, with one plane face, which resulted in the marked development of the hafted axe in the split haft. It is in this period too that we find the flint drill and pick well developed whilst the finer flaking has also made possible the flint knife.

Here again the distinction between the newly made knife of primitive man and the one which he had notched with constant use appears a comparatively slight one until we realise that the latter was the initiation of an evolutionary series beginning with the flint saw, and leading on to an entirely new phylum of man's tools, including a variety of implements of abrasion from the cross-cut of the lumberman to the file of the locksmith. So too in the rude scraper of Palaeolithic Man there is little to indicate its manifold derivatives, from the barrasquit d'Espourga, used to shave off the bark in the Landes, or the spokeshave of the wheelwright, to the fillisters and moulding planes of the cabinet-maker.

In the Neolithic period with its enormous advance in technique we find many of the dormant potentialities of the earlier types realised. The knives, picks, and saws had reached a high stage of development whilst the perfection of the boring implements is shown in the perforated stone axe-heads and the eyelet-holes of their bone needles. The hand-axe of earlier periods had already in the Palaeo-

lithic period given place to the hafted axe on the one hand and the spear-head, arrow-head, etc. on the other, all of which attained a much higher development in the Neolithic period.

To the biologist the implements of the Stone age in particular and in a lesser degree those of the Bronze and Iron ages are replete with examples of the importance in evolution of apparently trivial distinctions. As in animate objects, not all of these potentialities develop. In the earliest borers of the Chellaeen period we find the "ficron" with curved edges which become straight with the improved technique and finer flaking of the Acheulean type of borer. Thus a potentiality was lost, not to reappear, as a parallel development in metal, until quite modern times where the borer with the twisted edge finds expression in the high speed twist drill.

We see the forces of natural selection at work in the Neolithic period leading to the sifting out of specialised types in correspondence with the changed conditions consequent upon the replacement of Palaeolithic Man the hunter by Neolithic Man the agriculturalist. The hafted implement attains a high state of development and the so called "adze," probably used as a hoe, makes its appearance.

With the advent of metal an entirely new factor is introduced. It was as it were a profound mutation yet, as with plants and animals, the potentialities are not at first obvious and in the initial stages the evolutionary continuity appears to be scarcely affected by the change. Just as the first motor cars were mere caricatures of their horse drawn predecessors, so too the first bronze celts were mere replicas in metal of their stone prototypes.

The bronze axe was bound in the split short arm of the L-shaped haft and we find the capabilities of the new material first developed in the flange at all four edges, to prevent vertical movements, and in the elaboration of the "stop" or transverse ridge which held the axe from being driven back into the haft by the force of the blows. The final phases of this series are the socketed axe-heads with a vertical septum, clearly derived from completely bent-over flanges, and finally the socketed celt with no septum and a loop through which the binding thongs passed. The latter represents the end of a series which has become extinct but the less specialised palstave survives in the machete of modern days.

The chief effect of the introduction of metal is seen in the lengthening of the sword blades and in the manufacture of slender implements such as pins, needles, etc. The latter made of bone had however already appeared in Palaeolithic times and it is doubtful

whether the advent of metal at first added any new invention to man's implements though it greatly increased their efficiency (the fish-hook may perhaps have been an exception). On the other hand there was a great efflorescence of elaboration in the form of ornamentation. It is indeed quite late on in the period of metal that there appeared implements such as the scissors and its allies which metal alone made possible.

Parallel evolution and reversion have their examples in human implements as in biology. The hand-dagger of iron of the middle ages was essentially a reversion in metal to the hand-axe of the Palaeolithic period, and amongst the examples of incompletely perforated Neolithic axe-heads are specimens in which a central plug demonstrates the use of a type of boring implements which would appear to have lapsed but has reappeared in the "self-centering" bit of the present-day carpenter.

Turning to modern times we find that several of the types represented in the Stone age have become phyla, embracing a vast variety of forms exhibiting every degree of specialisation and complexity. We can see in the stone saw the origin of the band saw, circular saw, fret saw, rip saw, etc., and in the hand-axe the forerunner of the battle-axe, the hatchet, the adze, the hoe, etc.

Consideration of the more complex types of implements emphasises the fact that each new advance is in the nature of an increase in complexity and by a process of survival of the fittest this is followed by a period of simplification. Here too we see the analogy with biological phenomena as also in the origin of new types from the more generalised rather than the highly specialised examples.

When we attempt to classify human implements into species, genera, families, cohorts, phyla, etc., we are presented with much the same difficulties as in the animate kingdoms, despite the advantages which a knowledge of their chronological sequence confers.

How, for example, would one classify the "safety razor"? Is it a derivative of the ordinary razor and therefore to be placed in the same class as the knife and the sword or is it a lineal descendant of the plane and therefore of the hide-scraper of Palaeolithic Man? Or again, is the cooper's drawknife a derivative of the spokeshave which it morphologically resembles or is it a descendant of the hatchet to which it is perhaps more closely akin in function?

What may be regarded as degenerate types which differ in function whilst retaining morphological simplicity are well exemplified by the fish knife, the pallet knife, the butter knife, and many others.

The persistence of primitive types side by side with recent and more complex ones is illustrated by the manufacture of flint implements by the Tasmanian aborigines till well on into the nineteenth century. Moreover the cause of their survival is the same as that which accounts for the persistence of primitive biological types in the same area, viz. their isolation.

Even in the study of geographical distribution the analogy is not without value, for the origin and spread of man's inventions is well fitted to illustrate the importance of natural barriers or the relation between age and area.

If we consider the series of species presented by the genus *Alstroemeria* in which some have edge-on leaves occupying a profile position whilst others have leaves completely inverted involving a still further twist of the leaf base and accompanied by inversion of the internal structure, one cannot but ask why the apparently more fundamental change has been carried out (viz. complete inversion) rather than attainment of the same end by the apparently simpler course which an untwisting of the leaf base would have involved. For it can scarcely be doubted that *Alstroemeria* originally had a dorsiventral leaf with normal orientation. *Allium ursinum* illustrates the same point, whilst we see it again in the development of cladodes as leaves in place of renewed development of the scale leaves themselves.

Such examples, of which other cases might be cited, suggest a sort of momentum in evolution, a view which would harmonise with orthogenesis as conceived by Eimer. Such a standpoint seems more rational than the passive "law of loss" postulated by Dollo and others but, without arguing the point, it may be mentioned in this connection that man's implements often show a sort of momentum in their evolution which is perhaps not without significance for the students of phylogenetic sequence.

In the foregoing the writer has attempted nothing more than a mere adumbration of the possibilities of this analogy, but it will be evident that it is capable of development according to the needs of the teacher and his facilities for illustrating the analogy by means of suitable museum types. Its value as a teaching instrument largely depends on this latter, but in any circumstances one ventures to think that if students of biology occasionally examined anthropological collections from the evolutionary viewpoint they would find much to stimulate and more upon which to reflect.

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PERMEABILITY

By WALTER STILES

CHAPTER V

THE PERMEABILITY OF MEMBRANES

THAT colloids are generally incapable of penetrating into a colloidal system was mentioned at the end of the last chapter, and the incapacity of colloids to diffuse through colloidal membranes¹ is the phenomenon which led Graham to distinguish between colloids, substances which are incapable of passing through such membranes or which do so very slowly, and crystalloids, which pass through these membranes readily. By utilising this principle Graham was able to separate colloids from crystalloids, and so introduced the method of separation and purification of colloids known as dialysis.

The permeability of a membrane depends on the composition of the membrane, but the latter is not equally permeable to all substances. For example, caouchouc allows pyridine to pass through it while water is kept back. When a membrane is in contact with a solution the permeability may, and generally does, differ as regards the solvent and the solute. Thus parchment paper allows water to pass readily, but the solute in the case of an aqueous solution of sucrose diffuses through the membrane very slowly. In speaking of the permeability of a membrane it is thus necessary to define the system with which the membrane is in contact.

A membrane which allows a substance to pass through it readily is said to be *permeable* to the substance, while one which does not

¹ The term "membrane" may be limited to thin solid structures, while the term "film" is used for thin layers of liquid. In biology it is often difficult or impossible to distinguish between solid membranes and liquid films, and the term "membrane" will therefore be used to include films.

allow the substance to pass through it is said to be *impermeable* to the substance.

Although colloids generally cannot diffuse through membranes of colloidal substances, it must be realised that this distinction between colloids and crystalloids is by no means absolute. By varying the membrane and the substance, practically any degree of permeability may be found. Particularly interesting in this

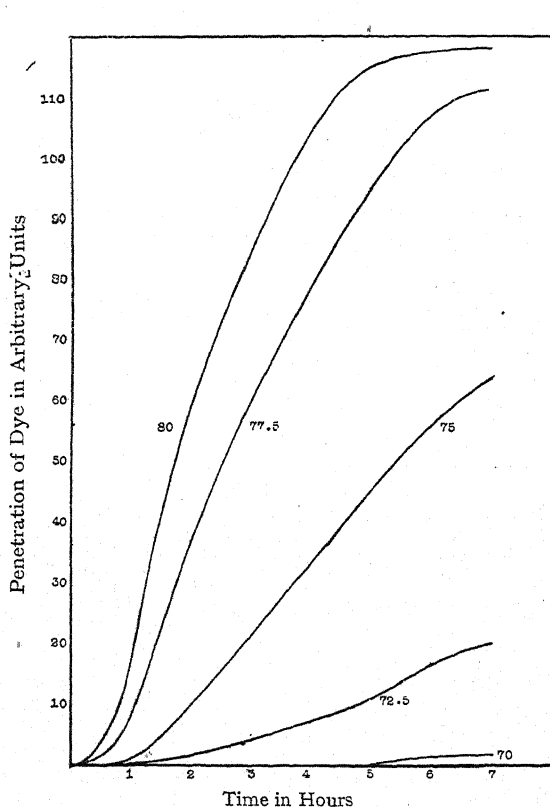


Fig. 3. Curves to illustrate the penetration of methylene blue through collodion thimbles of graded permeability. (Constructed from the data of W. Brown.)

respect are the observations of W. Brown (1915) who describes a method by which membranes of collodion may be prepared of any degree of permeability within certain wide limits. Brown's method consists in preparing completely air-dried membranes of collodion which are highly impermeable. The membranes are then immersed in solutions of alcohol in water for a suitable time. After washing in water the membranes then exhibit a per-

meability which increases with increase in the concentration of alcohol employed.

As an example of the degree of variation in permeability which may be obtained by this method an experiment made by Brown may be cited. Membranes in thimble form of different degrees of permeability were prepared as described by treatment with 70, 72.5, 75, 77.5 and 80 per cent. alcohol respectively. The thimbles were filled with a solution of methylene blue, and immersed in vessels containing distilled water. The amount of diffusion was estimated at daily intervals by colorimetric estimations of methylene blue in the external solution. The curves shown in Fig. 3 are constructed from the data given by Brown. They show what a wide range in permeability is obtained by grading collodion membranes in aqueous solutions of alcohol ranging in concentration from 70 to 80 per cent. As practicable membranes can be obtained by the use of alcohol solutions ranging in concentration from 0 to 97 per cent. it will at once be clear what an extremely wide range of permeability can be obtained at will. The following table gives the values found by Brown for what he calls the "alcohol index" of a number of substances. The alcohol index of a substance is defined as the number which represents the strength of alcohol required to produce a membrane which just prevents diffusion of the substance.

TABLE X

Alcohol Indices of a Number of Substances. (Data from W. Brown)

Substance	Alcohol Index
Water	0
Sodium chloride	0
Potassium permanganate	30-40
Picric acid	35-40
Copper sulphate	60-70
Potassium oxalate	60-70
Sodium sulphate	60-70
Bismarck Brown	65-
Methylene Blue	70-
Neutral Red	72.5-75
Safranin	75-77.5
Dextrin	85-87.5
Starch	90
Aniline Blue	92
Litmus (neutral)	93
Congo Red	96
Night Blue	>96

In a later paper Brown (1917) extends his method for obtaining membranes of graded permeability to other material (gelatine, agar-agar) and a number of grading liquids. Other methods of obtaining membranes of graded permeability are described by Bechhold (1907), Bigelow and Gemberling (1907), Schoep (1911) and Walpole (1915).

When a solution is in question a membrane may be equally permeable to both solvent and solute, or it may be impermeable to both. More frequently the permeability to the two components differs, and generally, in the case of solutions of crystalloids in water, the membranes are permeable to both water and the solute, and more readily to water.

The different rates of diffusion of solvent and solute through a membrane were shown in the first half of the nineteenth century by Dutrochet (1826, 1828, 1840, 1842) and Vierordt (1848) who examined the diffusion of aqueous solutions of salt through membranes of pig's bladder. The water passes through the membrane faster than the dissolved salt, this difference in rate depending on the nature of the salt, its concentration, and, as shown by Graham (1854), on the nature of the membrane.

Graham's discovery of the impermeability of certain membranes to colloids led Moritz Traube to search for a membrane which, while permeable to water, should be impermeable not only to colloids but to crystalloids. Such a membrane, permeable to the solvent but impermeable to any solute may be termed a *semi-permeable* membrane. Although a perfect semi-permeable membrane has never been manufactured, some of the precipitation membranes prepared by Traube (1867) are rather near approximations to one. The best known of these, and the one which has probably been most used, is that of copper ferrocyanide, which is obtained in the form of a gel when solutions of potassium ferrocyanide and copper sulphate come into contact. For most purposes these membranes are too delicate if unsupported, and so they are usually precipitated in the wall of a porous pot, a device due to Pfeffer (1877). Other precipitation membranes are those of glue-tannic acid, copper tannate, lead tannate, ferric ferrocyanide, copper silicate and tin silicate. These membranes, like the collodion membranes already mentioned, are graded in their permeability.

Tammann (1892) investigated the penetration of a number of salts through precipitation membranes of copper ferrocyanide and zinc ferrocyanide. He found the permeability of the two membranes was the same as regards the simple salts he investigated though not in regard to dyes. Traube (1867) had found the copper ferrocyanide membrane permeable to potassium, sodium and ammonium chlorides, and impermeable to barium chloride and nitrate, calcium chloride, potassium sulphate and ammonium sulphate. Tammann, on the contrary, found the membrane permeable to all these salts except calcium chloride. His findings in regard to permeability of the

copper ferrocyanide membrane to a number of salts of the alkali and alkaline earth metals are collected in the following tables.

TABLE XI

Permeability of Precipitation Membranes of Copper Ferrocyanide to a Number of Sulphates. (Data from Tammann)

Salt	Quantity passing through membrane
$(\text{NH}_4)_2\text{SO}_4$	small quantity
K_2SO_4 }	smaller quantity
Na_2SO_4 }	
Li_2SO_4	a trace
MgSO_4	none

TABLE XII

Permeability of Precipitation Membranes of Copper Ferrocyanide to Chlorides, Bromides and Nitrates of Metals of Alkalies and Alkaline Earths. (An asterisk indicates that traces only pass through the membrane.) (Data from Tammann)

Kation	Chloride	Anion Bromide	Nitrate
Ammonium	All penetrate the membrane in large quantities; much more so than the corresponding sulphates.		
Potassium			
Sodium			
Lithium			
Barium	perm.	perm.*	perm.*
Strontium	perm.*	perm.*	imperm.
Calcium	imperm.	imperm.	imperm.
Magnesium	imperm.	imperm.	imperm.

From quantitative investigations on the diffusion of acids through the copper ferrocyanide membrane, Tammann came to the conclusion that the rate of passage of the acid through the membrane was chiefly dependent on the degree of dissociation of the acid, the more dissociated the acid the more rapid its passage through the membrane.

An investigation by Walden (1892), the results of which were published almost contemporaneously with those of Tammann just quoted, agrees on the whole with these latter. Walden found, however, that membranes of copper ferrocyanide and zinc ferrocyanide had distinctly different permeabilities. Altogether the permeabilities of eleven precipitation membranes to a large number of acids and salts were examined. The membranes employed are recorded in the following table. All these membranes were impermeable to tannin, while all were permeable to the following anions when bound to alkali metal kations (K, Na, ?Li, NH_3): F, Cl, Br, I, CN, CNS, ClO_3 , ClO_4 , BrO_3 , IO_3 , NO_2 , NO_3 , formate, acetate, trichloracetate, iso-

butyrate, valerianate, salicylate, sulphate, thiosulphate, AsO_3 , B_4O_7 (the two silicate membranes were not examined in the case of the last four anions). The results with other ions are shown in Table XIII.

TABLE XIII

Permeability of Various Precipitation Membranes to Anions bound to Alkali Metals (K, Na, ?Li, NH_4) (p permeable, i impermeable).
(Data from Walden)

Membrane	Anion					
	PO_4	AsO_4	SO_4 (SeO_4)	CrO_4	P_2O_7	Oxalate Ferrocyanide Ferricyanide Cobaltocyanide Silicate
Glue-tannic acid	p	p	p	p	p	p
$\text{Ni}_3(\text{CoCy}_6)_2$	p	p	p	p	p	i
Ni_2FeCy_6	p	p	p	p	p	i
$\text{Co}_3(\text{CoCy}_6)_2$	p	p	p	p	i	i
Co_2FeCy_6	p	p	p (i)	i	i	i
$\text{Cd}_3(\text{CoCy}_6)_2$	p	i	i (p)	i	i	i
Nickel silicate	.	.	i	i	i	i
Cobalt silicate	.	.	i	i	i	i
$\text{Cu}_3(\text{CoCy}_6)_2$	i	i	i	i	i	i
Zn_2FeCy_6	i	i	i	i	i	i
Cu_2FeCy_6	i	i	i	i	i	i

In Table XIV are given Walden's results as regards the permeability of precipitation membranes to a number of positive ions bound (generally) to halogens.

The significant fact to be noted from Walden's results is that these membranes form a perfectly regular series in regard to their permeability to the substances examined, glue-tannic acid being the most permeable, and allowing the passage of everything investigated except alkali tannates and halides of (?) cadmium, zinc and manganese, while copper ferrocyanide is the least permeable, the other membranes being intermediate in their permeability.

From these results and those obtained with a large number of acids, Walden concluded that the penetrability of substances depends not so much on the number and weight of the atoms composing their molecules, as on the nature and arrangement of the constituent atoms. Thus sulphate and thiosulphate have the same number of atoms in the molecule, but precipitation membranes are much more permeable to the latter than to the former. The same is the case with sodium acetate and sodium oxalate. In general, while both electrolytes and non-electrolytes may be able to pass through a membrane, acids and salts containing at least one univalent ion diffuse through most easily. Potassium and ammonium chlorides, bromides and iodides pass through membranes with nearly equal ease. With other

TABLE XIV

Permeability of Various Precipitation Membranes to Positive Ions bound (in most cases) to Halogens. (Data from Walden)

Membrane	Permeable to	Impermeable to
Glue-tannic acid	Alkalies, Cu, Ba, Mg, Fe'', Ni, Co, Hg'', Pb, Al	?Cd; Zn, Mn''
Ni ₂ FeCy ₆	Alkalies, Mg, Ba, As	Ni, Co, Cu, Zn, Cd, Mn
Ni ₃ (CoCy ₆) ₂	Alkalies, Ba, As, Sb	Ni, Co, Cu, Zn, Cd, Mn
Co ₃ (CoCy ₆) ₂	Alkalies, Ba, Sb	Fe, Ni, Co, Cu, Zn, Cd
Cd ₃ (CoCy ₆) ₂	Alkalies, Ba, Sb	Cd, Co, Ni, Zn, Cu, Mn
Co ₂ FeCy ₆	Alkalies	Mg, Ca, Sr, Ba (slightly permeable), As, Co, Ni, Cu, Zn, Cd, Mn
Cu ₃ (CoCy ₆) ₂	Alkalies	Fe, As, Ca, Ba, Zn, Cu, Co, Ni, Mn, Cd
Zn ₂ FeCy ₆	Li, Na, K, Tl, NH ₄ , C ₂ H ₅ NH ₃ , (C ₂ H ₅) ₂ NH ₂ (slightly permeable)	Ca, Mg, As, Zn, Cu, Mn, Co, Ni, Cd, (C ₂ H ₅) ₃ NH
Cu ₂ FeCy ₆	Li, Na, K, Tl, NH ₄	Be, Mg, Cu, Sr, Ba, As

bases the diffusion of halides is greater the greater the atomic weight of the base.

The effect on permeability of the addition of neutral salts to acids varies according to the acid. In some cases such addition is without effect (for example with hydrochloric and trichloroacetic acids), in other cases there results a small hindrance to diffusion (as with sulphuric acid), while in other cases the addition of salts has a very significant effect (as with tartaric, glyceric and acrylic acids).

Tammann's observations on the penetration of 17 different dyes, including salts of colour bases, sodium salts of sulphonic acids, and acids, through three precipitation membranes, namely, glue-tannic acid, zinc ferrocyanide and copper ferrocyanide, showed seven exceptions to the regularity of the general order of permeability. Thus of the 17 dyes examined, eleven passed through the glue-tannic acid membrane, seven through the zinc ferrocyanide membrane and five through the copper ferrocyanide membrane. Nevertheless, fuchsin chloride, for instance, was able to penetrate the glue-tannic acid membrane and the copper ferrocyanide membrane, but not the zinc ferrocyanide membrane, although in general the zinc ferrocyanide membrane is more permeable than the membrane of copper ferrocyanide. An inverse case is that of cotton blue, to which the membrane of zinc ferrocyanide is permeable, but which can penetrate neither the copper ferrocyanide membrane nor the glue-tannic acid membrane, which is, in general, the most permeable of the three membranes examined.

It is to be noted in regard to the case of the permeability of the copper ferrocyanide membrane to fuchsin chloride, that according to Meerburg (1893) the dye is only able to penetrate the membrane until the latter is completely impregnated with dye.

Membranes then differ among themselves in regard to their permeability, and the permeability of any particular membrane is different to different substances. Generally the order of permeability to different substances is the same in the case of different membranes but there are exceptions to this rule. It would appear that membranes are much more permeable to halides than to sulphates and also considerably more permeable to salts of monovalent metals than to those of divalent metals. In some cases the permeability depends on the degree of dissociation of the substance but this is not by any means a universal rule.

Ostwald (1890) pointed out that in the case of a dissociated salt the membrane need only be impermeable to one ion of a salt in order to prevent both ions from passing, for on account of the electrostatic attraction of the oppositely charged ions the permeable ion will only travel to such a distance that its tendency to diffuse balances the electrostatic attraction.

It will be observed that very little quantitative work has been done on the permeability of membranes, and consequently exact data as to the influence of temperature and other factors on permeability are for the most part wanting. The influence of pressure on the passage of water through membranes has however been the subject of investigation by several workers, *e.g.* Schmidt (1856), Pfeffer (1877), Sebor (1904), Bigelow (1907) and Bartell (1911), from whose observations, and especially from those of Bigelow and Bartell, it appears that the rate of passage of water through membranes is proportional to the pressure. The membranes investigated by these different authors include various animal membranes (Schmidt), membranes of collodion and parchment paper (Bigelow), porcelain (Bigelow, Bartell), and copper ferrocyanide (Pfeffer, Sebor, Bartell).

The influence of temperature on the permeability of membranes of collodion to water at constant pressure was investigated by Bigelow and his results extended to porcelain by Bartell. The conclusion of these investigators is that the permeability expressed as the quantity of water passing through unit area of membrane in unit time, is given by the relation $M_\theta = M_0 (1 + 0.03368\theta + 0.000221\theta^2)$, where M_θ , M_0 are the permeabilities of the membrane at temperatures θ° , 0° respectively. As this formula is that connecting the viscosity of liquids at different temperatures the authors conclude that the

change in permeability of a membrane at different temperatures is nothing more nor less than a measure of the change of viscosity of the liquid.

There remain to be considered the various theories put forward to explain the differential permeability of membranes. There are two well-defined theories which are usually described as the sieve theory and the solution theory.

The sieve theory was first put forward by Traube (1867) who attempted to explain the semi-permeability of his precipitation membranes by supposing them to act as "atom-sieves" (more properly "molecule-sieves"), so that molecules below a certain size pass through, while those above this limiting size are kept back.

The experiments of Tammann and Walden already cited, as well as those of Kahlenberg (1906), do not wholly favour the sieve theory. Nevertheless, the theory has found in recent years a considerable number of supporters, *e.g.* Sutherland (1897), Perrin (1900). In an investigation on the passage of dyes through parchment paper Biltz (1910) found that the rate of passage of dye through the membrane depended very definitely on the dimensions of the molecule of dye. Thus when the number of atoms in the molecule did not exceed 45 the dye passed readily through the membrane; as the number of atoms in the molecule increases from 45 to 70 the rate of penetration through the membrane is slower and slower, and when the number of atoms in the dye molecule is greater than 70, the membrane is impermeable to the dye.

Also Bayliss (1915) points out that the difficulties in the way of the theory presented by Tammann's experiments in which the order of penetration of different substances varies with the membrane, can to a large extent be explained away.

Contrasted with the sieve theory is the solution theory of permeability, which may be traced back to the writings of Liebig (1849) and L'Hermite (1855). According to this theory substances pass through the membrane if they are soluble in the membrane, and are held back if they are insoluble in it. L'Hermite illustrated the theory with the "three liquid layers" experiments, one of which may be quoted as an example. Layers of water, castor-oil and alcohol were superposed in a cylinder, the castor-oil thus forming the layer separating water and alcohol. The alcohol is soluble in the castor-oil and passed through the middle layer composed of it (equivalent to a membrane) into the water. Similar experiments were performed with other systems of three liquids. The application of these experiments to the permeability of membranes

is obvious, and the solution theory of permeability has found many supporters, *e.g.* Nernst (1890), Kahlenberg (1906), and Overton (1897), to whose work more detailed reference will be made later.

From his work on the influence of pressure and temperature on permeability, Bigelow (1907) concludes that the passage of water through membranes of collodion obeys Poiseuille's formula for the movement of water through capillary tubes, and consequently that the passage of substances through such membranes is capillary in nature. The inconsistent results first obtained with porcelain were shown by Bartell (1911) to be due to mechanical clogging of the pores, a phenomenon which M. Traube thought he had been able to bring about in the case of precipitation membranes and which he adduced as evidence in support of the sieve theory. By clogging the pores of a membrane, the latter may become impermeable to substances to which it was formerly permeable. It has been pointed out by Bayliss (1915) that such clogging can also take place as a result of adsorption. The views of Bigelow seem at first sight to approach the sieve theory, but Bigelow shows that the capillary theory reconciles the opposed sieve and solution theories. In the case of porcelain the passage of water undoubtedly takes place through capillary pores, while in the case of water diffusing through collodion there is good evidence that the water passes through intermolecular spaces, *i.e.* dissolves in the membrane. But the phenomenon is essentially the same in the case of these and other membranes. Consequently Bigelow concludes that the rate of passage of liquids through molecular interstices is expressible by the same laws which formulate the rate of passage of liquids through capillary tubes.

This view, that capillary phenomena only differ in degree but not in kind, from chemical phenomena, can be traced back to L'Hermite.

Apart from the two definite theories of permeability here discussed, there has been suggested a third theory, which may be spoken of as the chemical theory, according to which the membrane is supposed to combine chemically with the substance to which it is permeable. This reaction is supposed to be reversible so that on the far side of the membrane the compound of membrane and diffusing substance breaks down with the result that the diffusing substance is set free on the far side of the membrane. This theory has been applied chiefly to cell problems, but has not found the support accorded to the sieve and solution theories.

(To be continued)

A STUDY OF SOME OF THE FACTORS CONTROLLING THE PERIODICITY OF FRESHWATER ALGÆ IN NATURE

BY WILLIAM J. HODGETTS, M.Sc.

(With 6 figures in the text)

VII. *SPIROGYRA*

NO less than 14 species of *Spirogyra* (namely *S. inflata*, *protecta* (= *calospora*), *colligata*¹, *longata*, *nitida*, *catenæformis*, *Grevilleana*, *condensata*, *maxima*, *bellis*, *varians*, *Jurgensii*, *mirabilis*, *Weberi*) have been observed in the pond during the period of observation, but although a few are hardy forms the majority are "spring annuals" (to use Transeau's term), and occur only during April—June, and are very rare or absent at other times.

As already stated (Section VI), *Spirogyra* predominates in the shallow-water marginal province, and rarely occurred in quantity in the central part of the pond during spring (February—May), culminating in a maximum generally in May, and rapidly declining in June, at the end of which month all species disappear, or become very rare, after producing zygospores. In the autumn (October—November) *Spirogyra* may again develop and become locally common in the pond, but is never so abundant as in the spring. The vernal phase was present in each of the four years, and was especially well developed in May 1919, when twelve species were observed, while an autumnal phase was present in 1918 and 1920, but was absent in 1919 (see below). The species which have appeared and conjugated in the autumnal phase are *S. inflata*, *protecta*, *colligata* and *varians*². The pronounced development of *Spirogyra* during the spring (March—May) has been noted by many observers (14, 16, 17, 18, 30, 31, etc.), and the less-marked autumnal phase has been recorded by Fritsch and Rich (14, 16, 17), Delf (12), as well as others.

The frequency-curves of *S. inflata*, *S. protecta* and *S. colligata* are given in Fig. 4. Of these three species the first two are the hardiest

¹ Hawkesley Hall pond is the original locality where this interesting species (see Hodgetts, 20) was found.

² Fritsch and Rich (17) give *S. varians* as an example of a species which "appears to be quite confined to the vernal phase." Conjugating specimens of *S. varians*, however, have been collected during the autumn months from several other ponds near Birmingham.

of all the species of the genus noted in the pond, *S. inflata* more particularly tending to be a perennial form. It will be seen that the general trend of the curves for *S. inflata* and *S. protecta* are the same; for instance, the maximum in October 1918 carries over both species through the winter into the following vernal phase, and a similar behaviour is shown by *S. colligata* in the very mild winter 1920-1921.

There is little doubt that the vernal maximum of *Spirogyra* (as Fritsch and Rich (16) concluded) is the result of the periodic recurrence of a group of certain factors, of which temperature is undoubtedly important. All the species (except *S. bellis*) of the genus noted in the pond appear to flourish best when the monthly mean temperature falls between 6° and 12° C., although the hardier species are able to tolerate much lower temperatures—for example, *S. protecta* was observed in January 1919 in a state of conjugation under a cover of ice 3-5 cm. thick. The higher summer temperatures are distinctly unfavourable, except perhaps in the case of *S. bellis*, which occurred locally during June-August, 1919, when the mean temperature of the air was 14° to 17° C. The autumnal maximum always falls when the temperature-conditions again become favourable, but of course some other condition may play the part of a limiting factor and prevent the development of this phase (cf. case of autumn 1919, discussed below).

Sunshine is another important factor, and a comparison of the curves of Fig. 4 with the sunshine-data (Fig. 2) is interesting. The sunniest month in each of the three complete years was May, and this is the month in which *Spirogyra* was at its best each year. In the very sunny month May 1919 all the species of the genus recorded for the pond (except *S. bellis* and *S. Grevilleana*) were present and produced zygospores; and of these *S. inflata*, *nitida*, *protecta*, *mirabilis*, *longata*, *colligata* and also *Grevilleana* were again present (and in conjugation) in May 1920; while in 1921 the vernal phase was fairly well developed (*S. colligata*, *protecta*, *inflata*, *mirabilis* again conjugating) and reached a maximum in the very sunny May of that year. The relation to sunshine is most obvious in the case of the more sensitive species (*S. catenæformis*, *condensata*, *Jurgensii*, *mirabilis*, *longata*, *maxima*, *Weberi*) which were confined to the vernal phase (maximum in May); while in the case of the hardier species (*S. inflata*, *S. protecta*, and in a lesser degree *S. colligata* and *S. varians*), as an examination of the curves shows, the relation is much less obvious. Thus the autumnal phase of *S. inflata* and *S. protecta* in 1918 was at a maximum in a dull month (October) although growth was most marked in the fairly sunny September; and in

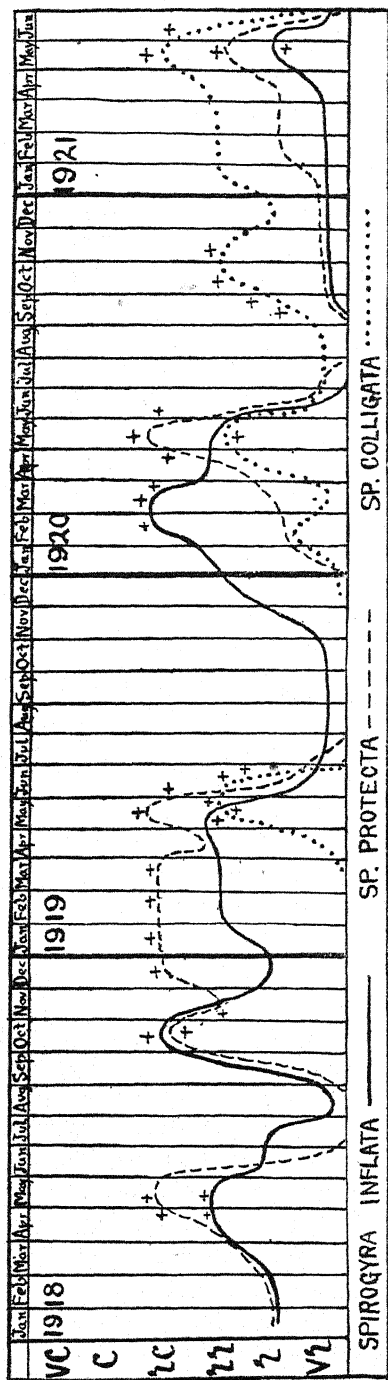


Fig. 4. Frequency-curves of three species of *Spirogyna*; vr = very rare, r = rare, rr = rather rare, rc = rather common, c = common, vc = very common; + denotes zygosporangium-production.

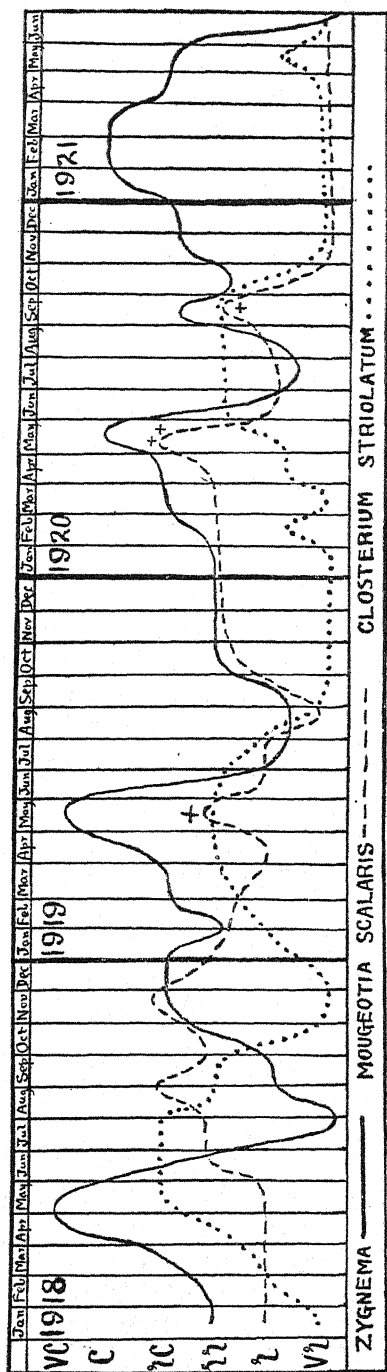


Fig. 5. Frequency-curves of *Zygnema* sp., *Mougeotia scalaris*, and *Closterium striolatum*.

1920 *S. inflata* reached its maximum and conjugated earlier than the other species, namely in February, and there was not much bright sunshine during January—February 1920.

An obvious relation is shown between the growth of *Spirogyra* in the pond and the amount of dissolved matter in the water (lowest curve Fig. 3). The greatest development of this genus coincided with a moderately low concentration of the water of 12 to 18 degrees, and undoubtedly this is the concentration which is most favourable, at any rate for the more sensitive species of *Spirogyra* (except perhaps *S. bellis*). During the spring of 1918 the concentration of the water was not determined, but in the autumn the concentration rose from 12 (September 21st) to 18 degrees (November 16th), and this corresponded to a pronounced autumnal phase in October (cf. Figs. 3 and 4). During the very pronounced *Spirogyra* maximum of May 1919 the concentration rose from 15 (May 17th) to 18 degrees (May 28th), but the further increase in the concentration during June resulted in the genus becoming rare.

An explanation is now forthcoming of the absence of an autumnal development of *Spirogyra* in 1919. During the summer of this year the water-level fell very low (see Fig. 3), and a very high concentration (reaching 30 degrees on November 22nd) resulted in the autumn, and there is every reason to believe that this was the particular factor which prevented the development of the genus during this season, especially as the meteorological conditions were favourable at that time. *S. inflata* is rather more tolerant in the matter of concentration (and also temperature and sunshine) than the rest of the species noted; the February maximum of 1920, for example, falling when the concentration was about 20 degrees, while the pronounced dilution of the pond during February—April 1920 affected this species but little (Fig. 4); and again in the early part of 1919 a similar disregard of a low concentration is shown by *S. inflata*, and also the hardy *S. protecta*.

The concentration-conditions during May 1920 were rather unfavourable, since after a fall to the very low concentration of 8 degrees on April 10th, and 9 degrees on May 8th, there was a very sudden increase to 18 degrees on May 29th¹. Now of the six species of *Spirogyra* present during April—May 1920 (the most abundant being *S. longata*, *protecta*, *colligata* and *inflata*), by the end of May two (*nitida*, *mirabilis*) had disappeared, two (*protecta*, *inflata*) had become very rare, while *S. longata* and *S. colligata* each remained "rather rare." It thus appears that a sudden increase in the con-

¹ See p. 159 for explanation of this sudden increase.

centration is distinctly unfavourable towards at any rate the more sensitive species of *Spirogyra*.

Fritsch and Rich (16, 17) were led to suggest that the normally recurring vernal phase of *Spirogyra* was dependent among other conditions upon the realisation of a certain degree of concentration of the water, and although they reached this conclusion mainly from a consideration of the rainfall-data alone nevertheless their conclusion has been amply verified in the case of Hawkesley Hall pond.

It is obvious that the different species of *Spirogyra* are not all alike in the way they react towards external conditions. In the case of the present pond the hardiest species observed is *S. inflata*, which tended to be almost perennial, and, as already stated, is more tolerant in the matter of concentration, temperature and bright sunshine than all the others noted; while *S. protecta* is also hardy but rather less so than *S. inflata*. These two species are also those which appeared first and developed best in the autumnal phase of *Spirogyra* (when this was present) and which often persisted in some quantity throughout the winter. A rather less tolerant species is *S. colligata*, which appeared in the autumnal phase of 1920, while *S. varians* (present and conjugated in September 1920, as well as in May 1919, and the spring of 1912) probably comes near *S. colligata* as regards its degree of sensitiveness. The position of *S. nitida* (present in autumn of 1918, but not conjugating) is rather doubtful, but it does not appear to be a very sensitive species.

Very sensitive species which developed only in the vernal phases (March—May) are *S. catenæformis*, *Grevilleana*, *condensata*, *Jurgensii*, *mirabilis*, *longata*, *maxima* and *Weberi*¹. These prefer a mean monthly temperature between 6° and 12° C., abundant bright sunshine and a moderately low concentration (between 12 and 18 degrees in the present pond) of the water. *S. bellis* developed rather exceptionally in the pond, but was confined to one small area, during June—August 1919, when the mean temperature was about 14°–17° C., and the concentration rising between 20 and 26 degrees, conditions which would undoubtedly preclude the growth of the majority of the species of the genus.

Pevalek (24) in his recent paper on the Algæ of N. Croatia found that the species of *Spirogyra* which appeared first in the annual cycle were the narrower ones with a single chloroplast and replicate end-walls, while in summer the wider species with several chloroplasts

¹ Doubtless these species might occur and even conjugate in the autumn if conditions were very favourable; Fritsch and Rich (17) noted *S. Weberi* in Abbot's pond in September 1905.

and plane end-walls developed, the order of appearance in the autumn being just the reverse¹. This, as is obvious from the work of others, is a fairly general rule, and appears to be correlated with the fact, pointed out by Fritsch (15), that the species of *Spirogyra* present in the tropics are mostly broad forms with two or more chloroplasts and plane end-walls. Transeau (28) has analysed this annual succession of species of *Spirogyra* in more detail.

Of course many other factors besides those treated of above influence the growth of Algæ in a pond, and one which is undoubtedly important at times is competition between the various species present. In the case of the present pond during May 1919, when at least twelve species of *Spirogyra* developed in the comparatively narrow zone of shallow water at the side of the pond, two of these (*S. protecta*, *S. longata*) were "rather common," six were "rather rare," one (*S. condensata*) was "rare," and the remaining three (*S. Jurgensii*, *mirabilis*, *Weberi*) "very rare." Mutual competition between these various species was, in this case, the particular factor which determined the extent of development of each.

VIII. ZYGNEMA AND MOUGEOTIA

A species of *Zygnema* (width of filaments 22–24 μ) is a perennial constituent of the algal flora of Hawkesley Hall pond, and, as mentioned in Section VI, is the dominant filamentous Alga in the central province of the pond during the spring, its maximum occurring usually at the end of April or in early May, although in 1921 the maximum fell exceptionally early (see curve in Fig. 5). The species was never observed to conjugate, and thus was not identified. Like *Spirogyra* it very rapidly decreased in amount towards the end of May, and was generally "rather rare" or "rare" by June. During the unfavourable summer-period it is rare and represented by more or less isolated and generally very short filaments, the cells of which, although comparatively thin-walled, are filled with abundant starch-grains, the whole contents of the cell being very dense, with an opaque granular appearance². Growth again becomes apparent in September, while during the autumn and winter the Alga is often "rather common" (1918, 1920); and the very mild winter 1920–1921

¹ Pevalék explains this by reference to the intensity of the light, but this factor alone does not appear to supply a satisfactory explanation.

² These short cell-rows with the cells densely packed with food-reserves are sometimes termed "cysts" (see West (30), p. 34).

resulted in the maximum of 1921 falling very early (January—March)¹.

This species of *Zygnema* is much more hardy than even the hardiest species of *Spirogyra*. It became really abundant only when the temperature was moderate (a monthly mean temperature between 5° and 12° C.). The falling off of the species which usually commences in May (April in 1921) is due apparently to the combined influence of several factors, of which the increased temperature and competition with *Edogonium* (see below, Section X) appear to be most active. The higher summer temperatures are particularly unfavourable, while the lower ones of winter may be tolerated: for instance in the winter 1919–1920 this Alga was “rather rare,” while in that of 1918–1919 it was “rather common,” apparently as a result of the mild November–December of 1918.

There seems little relation between the growth of *Zygnema* and the sunshine-data (cf. Figs. 2 and 5), unless it is that abundant bright sunshine is harmful. The pronounced development during March—April, and in October 1918, during April 1919 and 1920, and again in January 1921, in every case coincided with a rather dull or even a very dull month. The decrease in each of three complete years commenced in May (the sunniest month), so that excessive insolation may be a harmful factor, but it is difficult to separate its influence from that of the rising temperature.

A comparison with the concentration-curve in Fig. 3 shows that the most pronounced growth of this *Zygnema* coincides with low and moderately low concentrations of the water between 10 and 17 degrees, although it is clear that the form is very tolerant of other concentrations, both lower and higher. The conditions requisite for this species to conjugate were apparently not realised in the pond during the period of observation; or it may be that the species is one which conjugates very rarely, relying upon the production of “cysts” for tiding over unfavourable periods.

Filaments of *Mougeotia*, although almost always present in the pond, are generally rather scarce and more or less isolated. Two species (*M. scalaris* and *M. viridis*) have conjugated during the period of observation, but possibly other species have been present in a purely vegetative state in addition to these. The most abundant species of the genus in the pond was *M. scalaris*, a form which tends

¹ West (30) observes that the maximum growth of *Zygnema* in the upland lakes of the British Isles “usually occurs in the late summer and early autumn as the temperature is gradually declining.” This obviously is not applicable to the species now under consideration.

to be perennial, and the frequency-curve of which is given in Fig. 5. Five "maxima" of varying intensity are shown (three autumnal and two vernal), namely in August and November 1918, May 1919 and 1920, and September 1920, the species in all cases growing mainly in shallow water towards the margin of the pond.

Delf (12) observed that a wide *Mougeotia* (probably *M. scalaris*), in ponds on Hampstead Heath, showed an autumnal as well as a vernal phase; while the sterile species noted by Fritsch and Rich (17) in Abbot's pool also exhibited an autumnal development in certain years, but tended to have its maxima during December—February.

M. scalaris in Hawkesley Hall pond is a hardy form as regards its ability to withstand extremes of temperature, the five maxima occurring respectively at the following mean temperatures: 16.5°, 5.5°, 13.5°, 12° and 13.5° C. (monthly mean temperatures of the air). A comparison with the data given in Fig. 2 shows that the species does not require any considerable amount of bright sunshine for good vegetative growth, but that abundant bright sunshine and a moderately high temperature (monthly mean between 11° and 13.5° C.) are requisite for zygospore-formation. In May of both 1919 and 1920 there were slight maxima, and the species conjugated, and this month in both years was very sunny (Fig. 2). Zygospores were very scarce in the feeble maximum of September 1920 (a dull month); and the larger maxima of August and November 1918 (the former a rather sunny and the latter a dull month) were purely vegetative, the sunshine of August 1918 apparently not being sufficient to induce conjugation.

There is distinctive evidence, therefore, that the conditions favouring vegetative growth, and those best for the conjugation of *M. scalaris* are not identical; and further instances of the same phenomenon will be noted in the case of the Desmidiaceæ (Section IX). Fritsch and Rich (18) have pointed out the connection between bright sunshine and the fruiting of *Mougeotia parvula*, as well as other Algæ, thus confirming Klebs' conclusion by direct observation in nature. It is certain, however, that many Desmids (see Section IX), as well as some species of *Spirogyra*, can conjugate without the stimulus of abundant bright sunshine.

With regard to the influence of the concentration of the water on the growth of *M. scalaris*, the evidence does not seem to point to any particular degree of concentration being essential for growth, in fact the species is a very hardy one in the matter of concentration of the water. Thus between November 1919 and April 1920, when the concentration fell steadily from 30 to 8 degrees (see Fig. 3), the

species remained "rather rare" the whole time. Competition with other filamentous Conjugates (particularly *Spirogyra* spp.), which tend to grow mixed with *Mougeotia* in the shallow-water marginal region of the pond, is undoubtedly a potent factor in determining the extent of development of *M. scalaris* at certain times of the year.

Narrow filaments of *Mougeotia* were also generally present in the pond, but mostly only rare or isolated. Of these narrower forms *M. viridis* became "rather common" at the sides of the pond, and produced a fair number of zygospores, during September and October 1920, but declined during the following winter, and was not again observed. This slight autumnal development of *M. viridis* is interesting since, in this country, it is more particularly an early spring Alga. West (30) has pointed out that in the British Isles *M. viridis* is almost invariably the first species of the genus to fructify in spring—in the Midlands and Southern counties in March with a water-temperature of 5°–7° C. (*l.c.* p. 33). In the present pond it developed when the temperature was falling and the monthly mean (air-temperature) was between 13.5 and 10.5° C., and when the concentration was falling from 18 to 12 degrees, and the sunshine poor. The evidence is incomplete but it seems as though abundant bright sunshine, so essential for zygospore-production in the case of *M. scalaris*, can to a considerable extent be dispensed with by *M. viridis*; in which case an interesting parallel can be drawn between *Mougeotia* and *Spirogyra*, since the narrower species of the latter genus (e.g. *S. inflata*) require in a lesser degree the stimulus of bright sunshine in order to conjugate than do the wider species (cf. Section VII).

IX. THE DESMIDIACEÆ

Desmids are present in Hawkesley Hall pond all the year round, and are represented by a rather large number of species (see Section V). The following have been most abundant, and are those whose periodicities have been most thoroughly worked out: *Hyalotheca dissiliens*, *Sphærozosma granulatatum*, *Closterium acerosum*¹, *Cl. striolatum*, *Cl. moniliferum*, *Cl. Kützingerii*, *Cl. lineatum*¹, *Pleurotænium Ehrenbergianum*, *Cosmarium Botrytis*¹, *C. Regnellii*, *Staurastrum brevispinum*¹, *S. Dickiei*¹, *S. furcigerum*, *Xanthidium antilopæum*, *Arthrodesmus convergens*. Many of these tend to be scattered throughout the pond, although showing a preference for the deeper water, amongst the filaments of *Edogonium*, *Zygnema*, etc., but a

¹ These have produced zygospores.

few (*e.g. Closterium acerosum*) prefer the shallower water at the sides of the pond.

As regards their occurrence in the pond these Desmids may, for convenience, be divided broadly into two groups, the majority, however, falling in the second group.

GROUP 1 contains those Desmids which attain their maximum either in the spring or autumn (or have two maxima, one in spring and another in autumn). To this group belong *Hyalotheca dissiliens*, *Sphaerosma granulatum* and *Closterium acerosum* (Fig. 6).

GROUP 2 includes those Desmids which attain their maximum development at the end of summer or in early autumn (June—September), and includes all those of the list, with the exception of the three placed in the first group. *Closterium striolatum*, however, rather tends to form a connecting-link between these two groups.

From a consideration of the meteorological and other data it seems certain that the most important factors concerned in the development of all the Desmids of the pond are temperature and the concentration of the water. It is found by a comparison of the various curves that pronounced development of any particular Desmid depends on the conjunction of certain average temperature-conditions with a more or less definite concentration of the water, and that if only one of these conditions is favourable then the unfavourable one plays the part of a limiting factor and prevents the increased growth of the species.

Hyalotheca dissiliens (Fig. 6) is the hardiest of all the Desmids present, and a perennial constituent of the algal flora of the pond. It tolerates considerable extremes of concentrations (as in latter half of 1919, and first half of 1920), but nevertheless became more or less abundant only when the concentrations were moderately low (between 10 and 18 degrees—as during October—November 1918, April—May 1920, August—September 1920).

Again this species can withstand considerable variations in the temperature-conditions although distinctly preferring the moderate temperatures of spring and autumn; while the most unfavourable time for this Desmid, during the period of observation, was the hot summer of 1918. The lower temperatures of winter affect this Desmid in a much less marked degree than do the higher summer-temperatures. No relation to bright sunshine is apparent. The absence of a distinct vernal phase in 1920 was probably due to the marked dilution which the pond underwent during February—April of this year, favourable concentration-conditions apparently not arising until the higher unfavourable temperatures had set in—in short, the

low concentration of water possibly acted as a limiting factor during the early part of 1920.

Sphærozozma granulatum (Fig. 6) is a form decidedly more sensitive than *Hyalotheca*, as regards concentration of the water, but it may withstand somewhat higher temperatures, a monthly mean temperature between 6° and 13° C. apparently being most favourable. Higher summer-temperatures appear to have been harmful in 1918, but less so in 1919. The greatest observed development (October 1918) fell with the concentration rising gradually from 12 to 18 degrees, while the increase of this species during April—May 1919 also took place when the concentration was rising approximately between 10 and 18 degrees. The high concentrations attained in the latter half of 1919, and the sudden variations in the concentration during April—May 1920 (see Fig. 3), were much more unfavourable for this Desmid than they were for *Hyalotheca*, and caused the slight maximum of 1920 to fall late in the year, namely during August—October. It may be noted that the temperature-conditions during the cool summer of 1920 were never very unfavourable towards this species. No explanation can be offered for the non-appearance of *Sphærozozma* in the spring of 1921. There seems no relation to sunshine unless it is that continued bright sunshine is harmful (May 1918 and 1919), but it is difficult to separate the influence of this factor from that of the higher temperature.

Closterium acerosum (Fig. 6), in contrast to almost all the other Desmids of the pond, tends to thrive best in very shallow water at the margin of the pond. It is a typical vernal Desmid, although in 1918 it showed two maxima, one in spring and another during September—October. Vegetative growth was most active with a monthly mean temperature (of the air) between 7° and 13° C. (either falling or rising), and when the concentration of the water was between 10 and 16 degrees. During the autumn of 1919 the temperature was favourable (see Fig. 2), but the high concentration (cf. Fig. 3) was a limiting factor which prevented growth; and again the moderately high concentrations which prevailed during the dry spring of 1921, were doubtless the cause of the "maximum" of that season being very slight—in fact only in one month (April) did the concentration fall to a favourable degree, and this is the month in which the "maximum" occurred.

With reference to the conjugation of this species (which occurred in May 1919 and 1920), it is obvious that bright sunshine is a very important factor in bringing this about. During the maximum of October 1918, a month poor in sunshine, no conjugation was

observed—conditions were favourable only for vegetative growth; but in May 1919 and also 1920, both months with very abundant sunshine (cf. Fig. 2), zygospores of this species were locally common. A certain amount of vegetative growth generally precedes zygospore-production in all the Conjugates, and in April 1918 and 1921 this requisite degree of development in the case of the present species apparently was not attained. *Cl. acerosum*, in requiring abundant bright sunshine for conjugation, agrees with certain other Conjugates which have produced zygospores in the present pond (cf. *Spirogyra* spp., *Mougeotia scalaris*, *Cosmarium Botrytis*), although, as will be shown below, certain Desmids of the pond conjugated without the stimulus of bright sunshine.

Closterium striolatum (Fig. 5), as already mentioned, rather tends to form a connecting-link between the two broad groups into which the Desmids of the pond have been divided. The maximum of *Cl. striolatum* tends to fall when the average temperature-conditions fall between 7° and 14.5° C. (usually in spring and early summer, but throughout the whole of the mild summer of 1920), while the most favourable concentrations of the water lie between 12 and 20 degrees. Thus the species is adapted to rather higher temperatures, and distinctly higher concentrations than *Cl. acerosum*, although it can hardly be termed a summer-species. High temperature was probably the cause of the decrease of this Desmid during July—August 1918 (since the concentration remained favourable), but the decline during June—July 1919 was probably due more to the high concentration of the water than the temperature. The long-continued maximum of 1920 (up to September) was due to the fact that *both* temperature and concentration remained favourable during the cool summer of this year (cf. Figs. 2 and 3).

The Desmids of the second group—those whose development is favoured by the higher temperatures of summer and early autumn—now come under consideration. The periodicity-curves of three of these are given in Fig. 7. *Cosmarium Botrytis* (Fig. 7) shows a comparatively regular curve the maximum heights of which fall generally between May and September. The relation to the temperature (cf. Fig. 2) is obvious, monthly mean temperatures between 10° and 15.5° C. being necessary for good development. There is also a distinct relation to the concentration-curve (Fig. 3), the most favourable concentrations lying between 15 and 20 degrees. The rather early decline of this species towards the end of July 1919, at a time when temperature-conditions remained favourable, was obviously due to the high concentration of the water (rising to 27

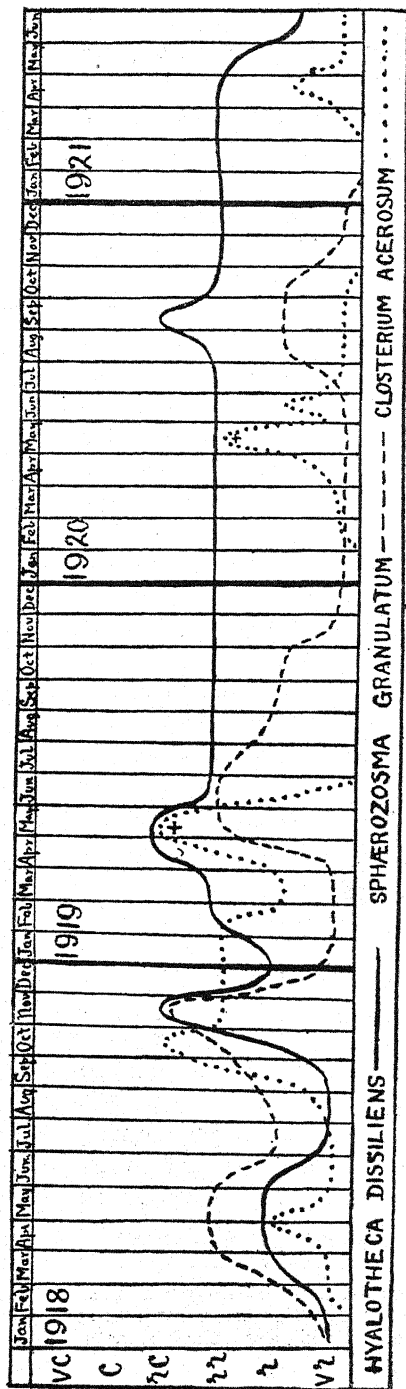


Fig. 6. Periodicities of three of the Desmids of Hawkesley Hall pond.

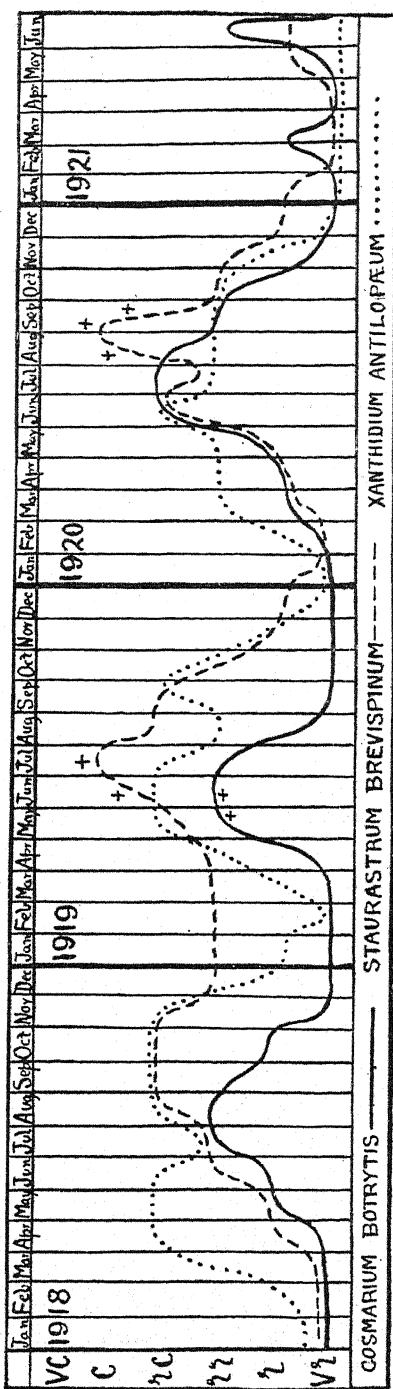


Fig. 7. Periodicities of three of the Desmids (summer-forms) of Hawkesley Hall pond.

degrees on August 22nd, 1919); and the decrease at the end of June 1921 was doubtless due to the same cause.

From a consideration of the sunshine-data it appears that only moderate amounts of bright sunshine are sufficient for good vegetative development, but that abundant bright sunshine is essential for conjugation. Zygosporos were observed (locally common) during May and early June 1919, and May 1919 was a record month for sunshine (Fig. 2). It is true that in 1918 the species attained its maximum in a sunny month (July), and no zygosporos were observed in that year; perhaps they may have been scarce and were overlooked. It is probable, therefore, that this Desmid agrees with *Closterium acerosum*, *Mongeotia scalaris*, *Spirogyra* spp., and doubtless many other Conjugates, in requiring abundant bright sunshine for zygosporos-production.

Cosmarium Regnellii gave a frequency-curve of almost exactly the same form as that of *C. Botrytis*, but was never represented by so many individuals as the latter species. *Cosmarium reniforme* was frequently observed in the samples from the pond but was generally very rare, although it showed a slight increase during the warmer months of the year.

Staurostrum brevispinum was one of the commonest Desmids observed in the pond, and gave a frequency-curve (in Fig. 7) the maxima of which tend to fall rather later than those of *Cos. Botrytis*, namely some time between July and early September, when the monthly mean temperature was between 13° and 16.5° C. The species, however, tends to persist in some quantity into the middle (1919) or even to the end (1918) of autumn, while it remained "rather rare" during the winter 1918-1919. It is thus a fairly hardy form, but attains its best development under summer-conditions. The most favourable degree of concentration of the water appears to lie between 14 and 21 degrees, and while lower ones are tolerated the higher concentrations apparently acted adversely in August and again in October 1919 (cf. with data in Fig. 3). There seems no relation between bright sunshine and vegetative growth.

Zygosporos¹ of this species were observed in the pond during June-July 1919, and again in August-September 1920, and, rather contrary to what might have been expected (cf. *Closterium acerosum* and *Cosmarium Botrytis* above), there seems little relation between bright sunshine and zygosporos-production in this Desmid. The 1920

¹ Apparently never recorded before since W. and G. S. West in their *Monograph of the British Desmidiaceæ* state (4, p. 146) that the zygosporos of this Desmid are unknown. They are 50-53µ diam., and have a thick, smooth, colourless wall.

maximum, especially, occurred after a month (July) poor in sunshine (cf. Fig. 2), yet zygospores were noted at the time to be "locally rather abundant." It is true that conjugation took place during the maximum of 1919 after two months of very sunny weather (cf. data in Fig. 2), but on that occasion zygospores were recorded as "very rare." The evidence therefore points to abundant bright sunshine not being essential for the conjugation of this species. It is interesting to note that West (30) was led to conclude that the precise conditions resulting in zygospore-formation in Desmids "are probably widely different for various species" (*l.c.* p. 35); and the present observations rather tend to confirm this view.

Two other species of *Staurastrum* (*S. Dickiei*, *S. furcigerum*) were present in the pond, but since their frequency-curves tend to follow very closely that of *S. brevispinum* they have not been reproduced here. Both tend to arrive at a maximum towards the end of summer, persisting as "rather common" until the middle of autumn, when they always decrease and remain "rare" (or "very rare") during the winter and early spring. All three species of *Staurastrum* were very tolerant of the high concentration (27 degrees) attained in August 1919, and all three remained "rather common" during August and September of that year. In this respect they are distinctly more hardy than the species of *Cosmarium* in the pond. This is quite the reverse of the conclusion arrived at by Fritsch and Rich (18) in the case of the Desmids of Barton's pond, since these authors concluded that the two species of *Staurastrum* (*S. tricornis*, *S. brevispinum*) were apparently much more sensitive to high concentrations of the water than the *Cosmarium* spp. and *Closterium* spp. present in that pond.

Zygospores of *Staurastrum Dickiei* were observed in the pond, but were scarce, on one occasion only, namely on August 7th, 1920 (when *S. brevispinum* was also in conjugation), and this was after a rather dull July, so that *S. Dickiei* appears to agree with the very closely allied *S. brevispinum* in not requiring abundant bright sunshine for conjugation to take place.

Xanthidium antilopæum (Fig. 7) is a perennial constituent of the algal flora of the pond, and is more abundant in the warmer months. A monthly mean temperature between 10° and 15.5° C. is essential for this species to become really common, although it may persist as "rather rare" at temperatures rather lower than these. The low temperatures of winter are decidedly harmful. The relation to the concentration of the water is again obvious (cf. Figs. 3 and 7), the most favourable concentration lying between 14 and 20 degrees. An

example of a high concentration causing a depression in the frequency-curve is apparent in July and August 1919; but the fall in the concentration during September 1919 caused the frequency-curve to rise again, although the further increased concentration (and also the falling temperature) during October soon caused the Alga to decline once more.

The following table gives the frequencies of the three remaining Desmids which were of importance in the pond.

Table¹ showing the Occurrence of Three of the Desmids present in Hawkesley Hall Pond during 1918-1921.

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<i>Pleurotænium Ehrenbergii</i>	1918	vr	vr	r	r	r	rr	rc	rc	rc	r	rr	rc
	1919	rr	rr	rr	rr	rr	rr	r	vr	vr	vr	vr	vr
	1920	vr	r	r	r	r	rr	rr	rr	rc	rr	rr	r
	1921	r	r	r	r	r	rr						
<i>Closterium moniliferum</i>	1918	vr	r	rr	rr	rr	rr	rr	rc	rc	rr	rr	rr
	1919	rr	rr	rr	r	rr	rr	r	r	vr	vr	vr	vr
	1920	vr	vr	vr	vr	r	rr	r	rr	rr	r	r	r
	1921	r	vr	r	r	r	r						
<i>Closterium lineatum</i>	1918	vr	vr	vr	vr	vr	r	rr	vr	vr	vr	vr	r
	1919	vr	vr	vr	r	r	rr	vr	vr	vr	r	vr	vr
	1920	vr	vr	vr	vr	vr	r	r	r	rr!	r	vr	vr
	1921	vr	vr	vr	vr	vr	vr						

All three it will be noted tend to have their maximum development during the summer or early autumn. Temperature seems to be the main determining factor, although sunshine may, in a lesser degree, be important. In general concentrations of the water between 14 and 20 degrees are most favourable; and, as with most of the other Desmids, the high concentrations attained in the second half of 1919 proved harmful. The curious secondary maximum of *Pleurotænium* in December 1918 was probably due to the fact that this month was abnormally mild (see Fig. 2). Both species of *Closterium* given in the table prefer distinctly higher temperatures than *Cl. acerosum*, the occurrence of which has already been discussed, although *Cl. moniliferum* (like *Cl. acerosum*) persisted as "rather rare" throughout the winter of 1918-1919.

The curious double zygospores of *Cl. lineatum* were observed in the pond (but were scarce) in early September 1920, and since

¹ The meaning of the symbols in this and in subsequent tables, and in the figures, is as follows: vr = very rare, r = rare, rr = rather rare, rc = rather common, c = common, vc = very common, - = absent; + (in the figures) and ! (in the tables) indicate sexual reproduction.

August 1920 had only a moderate amount of bright sunshine it seems that this species rather tends to follow *Staurastrum brevispinum* and *S. Dickiei* in not requiring abundant sunlight for conjugation, while differing in this respect (as in the matter of temperature) from *Cl. acerosum*. The same applies to *Cl. rostratum*, which was occasionally seen in the pond, although always very rare, the zygospores of which were observed in April 1919, a month which had only a moderate amount of bright sunshine. The Desmids of the pond which have conjugated thus fall into two classes, (1) those which require abundant bright sunshine before conjugation can take place (*Closterium acerosum*, *Cosmarium Botrytis*), and (2) those which apparently are able to conjugate given only a moderate amount of sunshine (*Staurastrum brevispinum*, *S. Dickiei*, *Closterium lineatum*, *Cl. rostratum*). The evidence in the case of the two species of *Closterium* in the second class, however, is not quite conclusive, since these Desmids were never present in any quantity.

X. THE ÆDOGONIACEÆ

Species of *Ædogonium* are always present in Hawkesley Hall pond, and as regards their periodicity can be sharply separated into two groups.

GROUP 1 contains probably only one species of *Ædogonium*, a wide species (width veg. fil. 30–35 μ), which since it has never reproduced sexually during the period of observation cannot be named, but which nevertheless is the most important of all the species of the genus observed in the pond, and is the form referred to simply as *Ædogonium* in the account (in Section VI) of the annual cycle observed in the central region of the pond. As already mentioned this species attains a huge maximum in the central deeper water of the pond generally during June–August, large masses of its filaments during these months occupying sometimes all the available free water-surface between the floating leaves of *Potamogeton natans* (cf. Section II).

GROUP 2 includes the rest of the species of *Ædogonium* occurring in the pond, and which even when taken all together were never as abundant as the species of the first group, and were never more than moderately wide (up to 20 μ wide). Their maximum growth was always attained during May or early June, a rapid decline taking place in the latter month after oospore-production. This group includes *O. cryptoporum*, *O. crispum*, *O. Borisianum*, *O. Braunii*, *O. echinospermum* and *O. rugulosum*. Several species of this group rather tended to show a preference for the shallower water at the

sides of the pond, often occurring in quantity round the submerged parts of rushes, etc., but *O. cryptoporum* and *O. Braunii* were also at times rather abundant in deeper water.

Dealing first with the wide species of the first group, the frequency-curve of which is given in Fig. 8, there is obviously a marked relation shown towards temperature (cf. Fig. 2), since the various maxima always coincide with the higher temperatures (a monthly mean between 12° and 16.5° C.), while the decline after a maximum is simultaneous with the lowering of the temperature at the end of summer or in early autumn. There was an exceptional smaller secondary maximum in the very mild October of 1920; while the maximum was attained very early (April) in 1921, probably owing to the warm spring of this year. There seems little or no relation towards bright sunshine, although growth was most marked in very sunny months in 1918 and 1920, yet in 1919 pronounced growth took place during March—April and again in July, and both these periods were poor in sunshine (cf. Fig. 2); and again the marked increase of the species in the abnormally mild January of 1921 was due solely to the rather favourable temperature, since this month was very dull.

An important factor affecting the vernal increase of this form is competition with *Zygnema* in the central parts of the pond. It has already been stated (Section VI) that the wide species of *Edogonium* replaces the *Zygnema* usually during June, so that competition between these two genera must be most marked in May and June; and it may be that this competition with *Zygnema* tends to prevent the species of *Edogonium* now under consideration from attaining its maximum early in the year, as do the narrower species of the genus in this pond. Although the *Zygnema* is a vernal type while the wide *Edogonium* is a summer-form, yet during the transition from spring to summer there is every reason to believe that there is active competition between the two, since both occupy identical positions in the pond, and the latter is not large enough for both to attain any great maximum at the same time.

A comparison of the frequency-curves of *Zygnema* (in Fig. 5) and the wide *Edogonium* (in Fig. 8) rather bears this out. In the first place the rise in the *Edogonium*-curve, just before a maximum, generally involves a fall in the *Zygnema*-curve; and further, in 1918 and 1919 at the periods of maximum development of the *Zygnema* the upward tendency of the *Edogonium*-curve is interrupted by a horizontal portion—a form of curve which indicates that some limiting factor has come into play, and is checking further growth. That this particular limiting factor is competition with the *Zygnema*

is indicated by the fact that upon its removal by the sudden decrease of *Zygnema* (usually in early June) the *Ædogonium*-curve again ascends to a higher level. In this connection the behaviour of the two curves in early 1921 is interesting. Owing to the very mild January of 1921 both Algae succeeded in becoming "common" by the end of that month, but mutual competition checked further increase until the end of March (shown by the flattening of both curves during January—March), when the *Ædogonium* assumed the upper hand and became "very common" during April, and, apparently as a consequence, the *Zygnema* suddenly decreased in amount.

Little relation is shown between the concentration of the water and the growth of this wide species of *Ædogonium*, although active development generally took place with moderately strong concentrations between 16 and 20 degrees (1918, 1920); but in 1919 the Alga was "very common" in August when the concentration was as high as 27 degrees (cf. Fig. 3).

Of the narrower species of *Ædogonium* (of Group 2) probably the most abundant was *O. cryptoporum*, the frequency-curve of which is also given in Fig. 8. The curves for the other species of this group, enumerated at the beginning of this section, are practically identical in form with that of *O. cryptoporum*, although the height of the May—June maximum varies with the different species (the result, doubtless, of mutual competition), the least abundant species being *O. Borisianum*, *O. Braunii*, and *O. rugulosum*. The spring-maximum (end of May and first half of June) was observed in each of the four years, and occurred with the average temperature of the air rising between 10° and 13.5° C., while the decline towards the end of June, after fruiting, coincided with the setting in of summer conditions. These species of the genus thus prefer a distinctly lower temperature than the wide species already considered.

The relation to the sunshine-data (Fig. 2) is very marked, the maximum always falling after a spell of very sunny weather. This agrees with the observations of Fritsch and Rich (18) who found that the maximum development of the species of *Ædogonium* in Barton's pond was "determined by the first month with plenty of bright sunshine." The second conclusion of these authors regarding these species, namely that "dilute water is more favourable to their development than concentrated water" (*l.c.* p. 78), is hardly confirmed by the present observations. The maximum of the species of *Ædogonium* which have fruited in Hawkesley Hall pond always occurred with medium concentrations between 16 and 20 degrees (cf. Figs. 3 and 8); and it may be noted that a sudden increase in

the concentration, as during May 1920 when the concentration rose from 9 to 18 degrees in the course of the month (cf. Fig. 3), did not check the development of these species.

It will be noticed that in regard to the species of the genus which were identified there is no evidence that the conditions requisite for good vegetative growth differ in any way from those essential for oospore-production, although, of course, such a difference may possibly exist. Undoubtedly the most curious feature about the occurrence of *Edogonium* in the pond was the independent way in which the wide sterile species continued its great maximum after the almost complete disappearance (during June) of the narrower species which had fruited. It may be that the wide species, having become perennial in the pond, has dispensed with sexual reproduction, or only resorts to it very occasionally.

The genus *Bulbochæte* is rather scantily represented in the pond, but *B. minor* has been observed during the spring of each of the four years, and although never abundant its frequency (Fig. 8) may be briefly referred to. Its maximum development fell usually during April—May (April—July in 1919), and it is undoubtedly a vernal form preferring average temperatures between 8° and 13° C., thus resembling very closely the species of *Edogonium* which fruited in the pond. The latter species have doubtless been partly responsible for the feeble development of *Bulbochæte* in the pond, since both generally grow together upon the same substratum (submerged stems of rushes, etc.) and are thus in competition with each other. In this connection it is noteworthy that *Bulbochæte* (mainly *B. minor*, but *B. intermedia* was also noted) was rather better developed in April—May 1918, as compared with the other years, and the narrower species of *Edogonium* were poorly represented during the spring of 1918. The dependence of *Bulbochæte* on abundant bright sunshine is very obvious (cf. with sunshine-data in Fig. 2), and this condition is very probably essential for the sexual reproduction of this genus.

XI. TRIBONEMA

This genus is represented in Hawkesley Hall pond by *T. bombycinum* (the type, and also its forma *minor*) and *T. affine*. As already stated in Section VI *Tribonema* is most abundant in shallow water near the sides of the pond during late autumn and winter, when it is often the best-developed filamentous Alga in the pond, although during the colder months *Spirogyra* (see Section VII) may be locally common, and *Microspora* (Section XII) may be more or less developed in places.

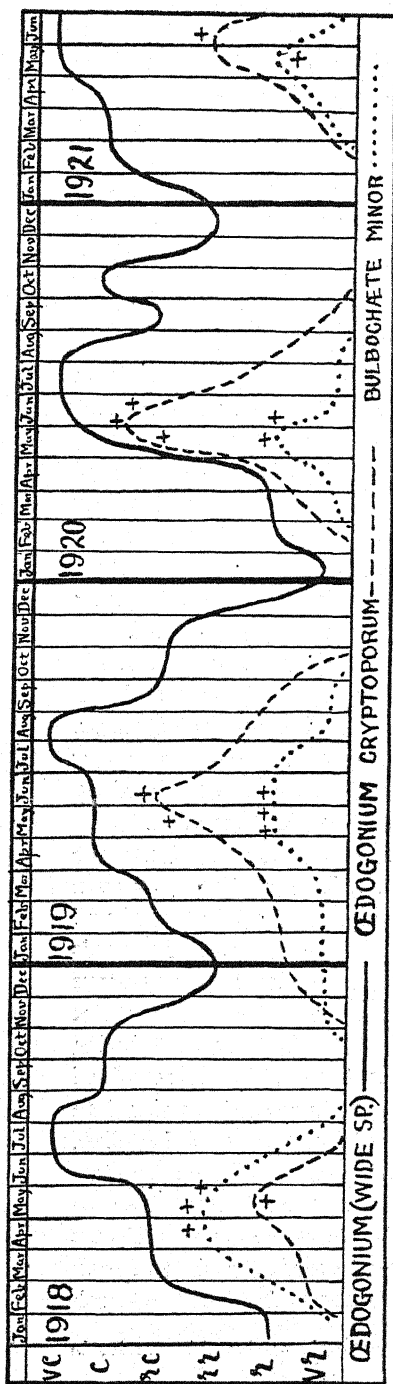


Fig. 8. Frequency-curves of three members of the CEdogoniaceae in Hawkesley Hall pond.

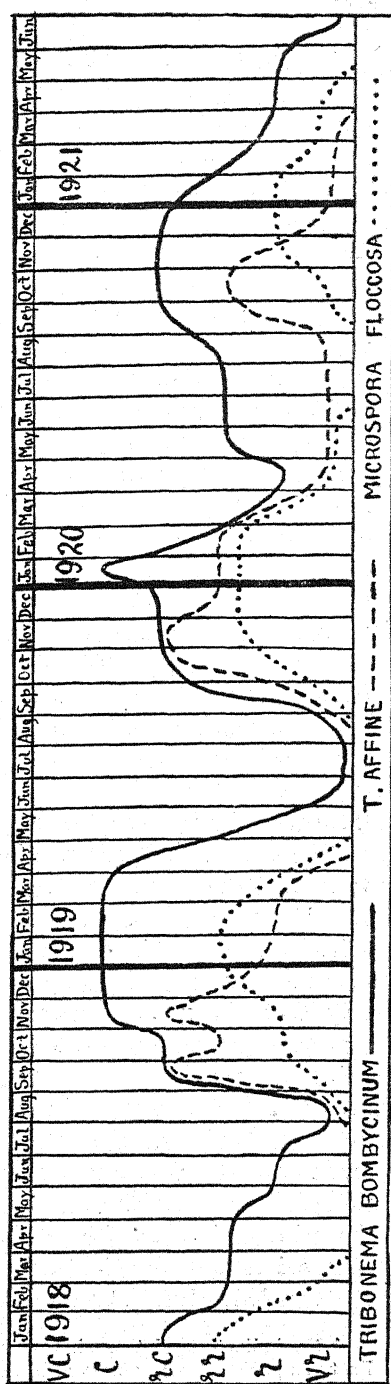


Fig. 9. Frequency-curves of *Tribonema* (two species) and *Microspora floccosa*.

The periodicity of *T. bombycinum* (type), given in Fig. 9, is represented by a fairly regular curve. Development commenced during September in 1918 and 1919, and the species rapidly reached a maximum which lasted throughout the following winter, the decline setting in usually in early spring (end of March in 1919, and January—February in 1921), while during the warmer months the Alga is usually rare¹. The species flourishes either when the temperature is falling (autumn), or when it remains persistently low or rather low; while the rising temperature of spring seems very unfavourable. A marked feature of its frequency-curve is the fact that during the notoriously cold summer of 1920 the species persisted as "rather rare," whereas it was "very rare" in the hottest months of the two preceding summers.

A marked antagonism appears to exist between the growth of *T. bombycinum* and bright sunshine (cf. data in Fig. 2), the maxima of the frequency-curve of this species always occurring when the daily mean of bright sunshine during the month is less than three hours; although, as with many other species, it is difficult to separate the influence of sunshine and high temperature. The adverse influence of bright sunshine on the development of several members of the Heterokontæ was emphasised by Fritsch and Rich⁽¹⁸⁾, and the present observations tend to confirm this in the case of *Tribonema*. It was noted that the species of this genus showed a preference for those parts of the margin of the pond which were most shaded by the leaves of grasses, and the various marginal Phanerogams.

There seems almost no relation between the concentration of the water and the growth of *T. bombycinum*, low, moderate, or even very high (October—November 1919) concentrations apparently all being the same to this species. The very high concentrations towards the end of 1919 (see Fig. 3) apparently depressed its frequency-curve only to a slight extent. Some explanation seems necessary to account for the early decline of the species in late January and during February 1920, at a time when temperature-conditions remained favourable (see Fig. 2). February 1920 was somewhat sunnier than usual, but hardly sufficiently so to account for the observed decline, and a probable adverse factor was the sudden fall in the concentration of the water during February—March 1920 (Fig. 3), although the evidence is not quite conclusive. *T. bombycinum* was always observed to form abundant aplanospores towards the close of each of its periods of abundance (during February—April 1919, March

¹ No indication of two phases, such as were shown by this species in Barton's pool (18), was observed in the present pond.

1920, February 1921.) Generally two rounded aplanospores were formed in each cell and the filaments broke up into H-shaped pieces in order to set them free. In addition to forming these aplanospores, however, the species generally persisted through unfavourable periods by means of isolated vegetative filaments.

T. bombycinum f. minor was quite as frequent in the pond as the type form, and its periodicity was worked out separately, but since the resulting curve is almost identical with that of the type (given in Fig. 9) it has not been reproduced here. Undoubtedly this form is influenced by external conditions exactly in the same way as the type, and the two were generally found intermixed, so that the above remarks concerning *T. bombycinum* apply with equal force to its forma *minor*. Doubtless between such closely allied forms growing together competition must be very active. No aplanospores were ever observed in the case of *T. bombycinum f. minor*, the Alga always tiding over unfavourable seasons by means of isolated filaments.

T. affine was in general decidedly less abundant than *T. bombycinum*, with which it was usually mixed. Its frequency in the pond (given in Fig. 9) very closely resembles that of *T. bombycinum*, activity commencing during the falling temperature of early autumn, and a maximum reached in October or November. Other factors, apart from temperature, however, caused the behaviour of this species to vary somewhat during the winter months, the most important probably being the concentration of the water. While *T. affine* apparently agrees closely with *T. bombycinum* in the matter of temperature and also sunshine, it reacts somewhat differently in regard to the concentration of the water. On two occasions (November 1918 and 1919) the position of the maximum on the *T. affine* curve coincides with the highest concentration reached during the autumn and winter months (cf. data in Fig. 3), and on one of these occasions (November 1919) the concentration was the highest (30 degrees) observed during the period of observation. In *T. affine*, therefore, we have an Alga which has its period of development (autumn—winter) determined mainly by the low temperature and scarcity of bright sunshine, although the actual position of maximum growth in this period is conditioned by the concentration of the water, the "maximum" coinciding with the highest concentration attained during the season.

There seems little doubt from the above observations that *T. affine* is able to utilise directly the organic peaty compounds which were abundant in the pond during times of high concentrations (cf.

Section IV), although culture experiments would perhaps be necessary to prove the matter definitely. Others have noted this preference of *T. affine* for peaty water: West for example (31) stated that *T. affine* "is not uncommon in peaty ponds and ditches" (*l.c.* p. 430). Doubtless the fact that this species has usually only two small, very pale chromatophores lends support to the view that its nutrition in nature is mixotrophic (cf. *Chlamydomonas*, Section XVII, and the Cyanophyceæ, Section XVIII).

There was doubtless a certain amount of competition between the two species of *Tribonema* in the pond, as a comparison of the curves suggests, and in the following section it will be seen that there was also fairly obvious competition between *Tribonema* and *Microspora*—genera showing very similar periodicities and usually found growing together in the shallow-water marginal region of the pond. Competition between these two genera was also noted by Fritsch and Rich (18) in Barton's pond.

XII. MICROSPORA

This genus was never very abundant in the pond, although represented by two species, *M. floccosa* and *M. stagnorum*, the former being the more important. From the frequency-curve of *M. floccosa*, given in Fig. 9, it will be seen that the maxima tend to fall regularly in the coldest months of the year, when the monthly mean temperature is between 1.5° and 5.5° C., and there is no doubt that a low temperature is the most important factor determining the occurrence of this species. This agrees with the observations of Fritsch and Rich (18) who found that the maximum development of *M. amœna* var. *gracilis* in Barton's pond was determined by the lowest winter-temperatures. The rather late attainment of the maximum of *M. floccosa* in 1918, as compared with 1919, is correlated with the fact that November and December 1918 were rather mild (cf. Fig. 2); while the general scarcity of the species in the winter 1920–1921 was doubtless due to this season being very mild.

As will be seen from Fig. 9, *Microspora* and *Tribonema* tend to arrive at a maximum at the same time of the year, and since the two generally grew together it is fairly obvious that the former genus suffered by competition with the latter. Exactly why *Tribonema* always succeeded in getting the upper hand, and operated as a limiting factor checking the growth of *Microspora*, it is difficult to say; the actual conditions favouring the last-named genus would have to be studied more fully in some pond where its development is not restrained by competition with other species. The case of *Micro-*

spora and *Tribonema* in this pond is somewhat analogous to that of *Bulbochete* and *Edogonium* (see Section X).

Certain filaments of *M. floccosa* always passed into the akinete-condition towards the close of the vegetative period (usually during March), persisting in this way throughout the summer months. *M. stagnorum* generally occurred mixed with *M. floccosa*, and like the latter is a cold-water species, although it is clear that it was affected very adversely by competition with *M. floccosa* and also *Tribonema*. It exhibited a slight maximum in October 1918 (mean temperature 9° C.), but the abundance of *Tribonema* (and *M. floccosa*) caused a quick decline in early November. A further maximum occurred in November—December 1919, the development of the species coinciding with that of *M. floccosa*. Both the latter species and *M. stagnorum* show no relation to the concentration of the water.

Here it may be noted that it seems a general rule that the occurrence of any Alga which is struggling to develop under conditions in which it is adversely influenced by competition with other species (of animals or plants) will tend to show little or no relation to such factors as concentration of the water and bright sunshine (although it may still exhibit some relation towards temperature), since it is *competition* which is the particular factor which sets a limit to the growth of the species, and restrains its development even though all other factors may be favourable for its increased growth. In small bodies of water, such as Hawkesley Hall pond, and with numerous species present; it is very probable that more or less competition is going on at all times of the year.

XIII. VAUCHERIA

This genus was represented in the pond by two species, *V. avera* and *V. sessilis*, although the latter was present in quantity only upon one occasion. The occurrence of *V. avera* is given in the following table:

Table¹ showing the Occurrence of *Vaucheria avera* in Hawkesley Hall Pond during 1918–1920.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1918	—	—	—	—	—	—	—	—	—	—	—	—
1919	rc!	rr!	rr!	vr!	vr	—	—	—	—	vr	vr	c!
1920	rc!	rc!	r!	vr	vr	—	—	—	—	—	—	—

¹ For symbols see footnote on p. 210. An exclamation mark denotes oospore-production.

V. aversa tends to develop best in fairly shallow water near the sides of the pond, and generally forms more or less definite communities, often almost unmixed with other filamentous species, although sometimes growing in company with *Tribonema bombycinum*. It is a form which prefers cold or moderately cold water, the most favourable temperature apparently being a monthly mean between 4.5° and 8° C., and thus development tends to fall in autumn or winter, while the species may persist into the following spring (see table above). It was most abundant during late December 1918 and early January 1919, when the concentration of the water was rather low, namely 13 degrees (cf. Fig. 3); while a smaller maximum occurred during January–February 1920, with the concentration in the neighbourhood of 20 degrees. The evidence, although not conclusive, points to moderate or rather low concentrations being preferred. During the very high concentrations at the end of 1919 (Fig. 3) the species was very rare, and did not commence development until late December, when the concentration was falling very rapidly.

Bright sunshine is certainly not necessary either for good vegetative growth or for the development of sexual organs in *V. aversa*, since both the maxima coincided with periods very deficient in sunshine. *V. aversa* thus differs from many species of *Spirogyra* and *Edogonium* (and apparently also from *V. repens*, according to the experimental work of Klebs) in fruiting without the stimulus of bright sunshine¹.

From the usual mode of occurrence of *Vaucheria* it appears that abundant aeration of the water is essential for good development, and this is borne out by the present observations on *V. aversa*, since the various maxima of this species always followed a month of heavy rainfall, when there would be a marked increase in the gaseous content of the water, since the rain of course would bring in a supply of dissolved gases from the atmosphere, and the prevailing low temperature would favour the continuance of this condition. The slight vegetative development of *V. aversa* which occurred during September–October 1918 (cf. above table) coincided with a phenomenally wet September (see rainfall-data in Fig. 3); while December 1918 was the second wettest month of the year, and the species again increased during that month. December 1919 was also the second wettest month of 1919 and *V. aversa* showed increased development during that month.

¹ Fruiting specimens of *V. aversa* (and *V. geminata*) have been collected on several occasions from small pools near Birmingham, and always in the colder months of the year when sunshine is deficient.

Vaucheria sessilis has been found in the pond on three occasions, namely October 1919 (rare), September 1920 (very rare), and April—May 1921 (rather rare) and was absent, or occasionally perhaps rare and vegetative only, at other times. Although the evidence is insufficient this species apparently differs in its requirements from *V. aversa*. During its period of greatest development in the pond (April—May 1921) the average temperature of the air was between 7° and 11° C., the concentration of the water was rising between 15 and 18 degrees, there was abundant sunshine, and rain was very deficient during the preceding three months, so that the aeration of the water was probably poor.

XIV. APHANOCHÆTE AND DRAPARNALDIA

Aphanochæte repens and *Draparnaldia glomerata*, although not very important constituents of the algal flora of the pond may be briefly referred to. The occurrence of the former species is given in the following table:

Table showing the Occurrence of *Aphanochæte repens* in Hawkesley Hall Pond during 1918–1921.

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1918	vr	vr	r	r	rr	r	vr	vr	r	rr	rr	r
1919	r	rr	rr	rc	rr	rr	rr	vr	vr	vr	vr	vr
1920	vr	vr	vr	vr	rr	r	r	vr	vr	rr	rr	rr
1921	rc	rc	rc	rr	r	r						

Aphanochæte repens was observed in the pond as an epiphyte upon the wide sterile species of *Edogonium* (rarely upon the narrower species, and very rarely upon *Microspora*) which generally formed such a conspicuous feature in the central parts of the pond during the summer months (cf. Section X and the curve of this species in Fig. 8). The occurrence of *Aphanochæte* it will be noted shows only a slight relation to the periodicity of its "host," since although the maximum development of the epiphyte occurred either in May (1918, 1920), April (1919), or during January—March (1921), which in each case coincided with the vernal increase of the species of *Edogonium* in question, yet *Aphanochæte* always decreased in amount long before the *Edogonium* commenced to decline. In fact *Aphanochæte* is a spring-form (although sometimes showing a certain amount of development in the autumn, as in 1918 and 1920), preferring moderate temperatures (a monthly mean between 4° and 11° C.). The higher temperatures of summer are very unfavourable

towards its growth. In the spring-like winter of 1920-1921 it will be noticed that the species reached its maximum very early (January 1921).

Aphanochaete prefers low or moderately low concentrations between 10 and 17 degrees; high or very high concentrations (as in second half of 1919) are particularly harmful. Of course the presence of a suitable "host" upon which to grow is essential for this species, but in Hawkesley Hall pond fortunately the wide species of *Edogonium* was generally so abundant (except in the colder months) that lack of a suitable "host" was rarely a factor limiting the growth of this epiphyte¹. But such a condition obtained in April 1920, in which year the wide *Edogonium* commenced development much later than usual (see curve of this species in Fig. 8), and when it did commence rapid growth at the end of April and early May the *Aphanochaete* developed *pari passu* with it until the end of May, when the higher temperature stepped in and caused the epiphyte to decline.

Draparnaldia glomerata attained only one maximum in the pond during the period of observation, namely in January 1920 (it was "rather common"), when the mean temperature of the air was about 5° C., and the concentration of the water rather high (falling, and between, 24 and 20 degrees). The species is undoubtedly a cold-water type, and tends to have its maximum development during the winter months, as noted by Delf (12), and also by the present author in the case of certain other ponds near Birmingham. Why it did not appear during the winter 1918-1919 cannot be decided from the data available; perhaps the prevailing low concentration of the water at that season had something to do with it, or perhaps the species was suppressed by severe competition. Its absence in the winter 1920-1921 was perhaps due to the very mild conditions which obtained during that season. During February 1920, towards the close of its period of development in the pond, the species produced abundant aplanospores.

XV. NITELLA AND COLEOCHÆTE NITELLARUM

Nitella flexilis was observed in Hawkesley Hall pond from 1919 to 1921, and it was always accompanied by *Coleochaete Nitellarum*, an endophytic species which grows between the lamellæ of the outer cell-walls of *Nitella* (and *Chara* spp.). The periodicities of these two species are given in the table below.

¹ Compare in this respect the relation between *Nitella* and *Coleochaete Nitellarum* in the pond, discussed in the next section.

Table showing the Occurrence of *Nitella flexilis* and *Coleochæte Nitellarum* in Hawkesley Hall Pond during 1919-1921.

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<i>Nitella flexilis</i>	1919	—	—	—	vr	r	rc!	rc!	rr!	rr!	rr	rr	vr
	1920	vr	vr	vr	rr!	rr!	r!	vr	vr	rr!	rc!	rc!	rc
	1921	rc	rc	rr	rr	vr	r						
<i>Coleochæte Nitellarum</i>	1919	—	—	—	vr	vr	rr!	rc!	rr!	rr!	r	vr	—
	1920	—	—	vr	r	rr!	r!	vr	—	r	r	r	rr
	1921	rr	rr	rr	r	vr	vr						

Nitella flexilis developed rather irregularly in the pond, and in the form of more or less local patches growing from the bottom in places where the water was only moderately deep. Its growth was undoubtedly greatly hindered by the extensive development of *Potamogeton natans* during the summer (cf. Section II), probably owing to the fact that the floating leaves of this plant cut off considerable light from the bottom of the pond; and it was observed that the patches of *Nitella* were always confined to those parts of the water where the floating leaves of *Potamogeton* happened to be poorly developed or absent altogether. No very close relation to the temperature is apparent; the first maximum occurred when the monthly mean temperature fell between 13.5° and 14.5° C., the second with a rising spring-temperature, while the rise to the third maximum coincided with a falling temperature in autumn, although the species persisted in quantity throughout the succeeding mild winter. Apparently a moderately high temperature (a monthly mean between 9° and 14° C.) is best for good vegetative growth, although somewhat lower ones are tolerated.

A rather close relation is shown between the vegetative growth of *Nitella* and the sunshine-data, while abundant bright sunshine is particularly essential for the development of sexual organs in this species. Thus the increase to the first maximum, in 1919, was preceded by two months of very sunny weather, while development towards the end of March 1920 coincided with a sunny March (see data in Fig. 2). Sexual organs were formed in abundance in the case of the maximum of 1919 and the first maximum of 1920, when the species had the stimulus of abundant sunshine (see Fig. 2), but were scarce during September—November 1920 (dull months), and absent altogether during the remaining months (which were dull, but mild) of this long-continued third maximum. There is no doubt therefore that oospore-production in *Nitella flexilis* is favoured by abundant bright sunshine.

No relation can be demonstrated between the concentration of the water and the growth of *Nitella*. The marked development during May—June 1919 coincided with a concentration rising from 18 to 20 degrees, but the high concentration (27 degrees) of August 1919 apparently affected this species very little, while the slight maximum during April—May 1920 occurred with an extremely low concentration (cf. Fig. 3).

With regard to the endophytic *Coleochaete Nitellarum* it will be noted at once on reference to the above table that the growth of the *Coleochaete* followed almost exactly that of the *Nitella*. This means that the amount of the latter plant present in the pond always operated as a limiting factor and restrained the growth of the endophyte, a condition of affairs just the reverse of that which obtained between *Aphanochaete* and the wide *Edogonium*, discussed at some length in the last section. With the two last-named species it was noted that the epiphyte had a periodicity very different from that of its "host," and this might, or might not, have been the case with *Coleochaete Nitellarum* and *Nitella* had the latter plant been more abundant in the pond. Thus an epiphyte is unable to exhibit, so to speak, its true periodicity when its growth is held in check by the scarcity of a suitable "host," and under such conditions its occurrence will tend to follow very closely that of its "host," although this does not necessarily mean that the two are influenced in the same way by external conditions.

It may be pointed out that abundant bright sunshine is also essential for the development of oospores in *Coleochaete Nitellarum*. This is shown by the fact that in 1920, although development commenced in March, no oogonia were observed until the end of May (the first very sunny month); and further by the fact that no oogonia were observed in the case of the third maximum (September 1920—March 1921) which developed during a dull period.

XVI. THE PROTOCOCCALES

The principal members of this group which have been at all abundant in Hawkesley Hall pond are *Dictyosphaerium Ehrenbergianum*, *Ankistrodesmus falcatus*, *Scenedesmus denticulatus* var. *linearis*, *Pediastrum Boryanum* and *P. tetras*. The first-named species was the most abundant, and its occurrence is given in Fig. 10, while the table below gives the periodicities of the remaining four species.

Table showing the Occurrence of Four Members of the Protococcales in Hawkesley Hall Pond during 1918-1921.

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
<i>Ankistrodesmus falcatus</i>	1918	vt	vt	vt	r	rr	rc	rr	rr	r	vt	rr	r
	1919	r	vt	vt	vt	vt	vt	rr	rr	r	r	r	r
	1920	vt	vt	vt	vt	vt	vt	r	rr	rr	rr	vt	vt
	1921	vt	vt	vt	vt	r	r						
<i>Scenedesmus denticulatus</i> var. <i>linearis</i>	1918	vt	vt	vt	r	rr	rr	rc	rr	r	r	vt	vt
	1919	—	—	vt	vt	vt	vt	rr	rr	r	r	r	r
	1920	vt	vt	vt	vt	vt	r	rr	rc	rr	r	vt	vt
	1921	vt	vt	vt	vt	vt	vt						
<i>Pediastrum Boryanum</i>	1918	vt	vt	vt	vt	vt	vt	rr	rr	rr	rr	r	vt
	1919	vt	vt	vt	vt	vt	vt	vt	r	rr	r	vt	vt
	1920	vt	vt	vt	r	r	r	r	rr	rr	rr	r	r
	1921	vt	vt	vt	r	r	r						
<i>Pediastrum tetras</i>	1918	—	vt	vt	vt	r	r	rr	r	r	r	vt	vt
	1919	vt	vt	—	—	vt	vt	vt	r	r	r	vt	vt
	1920	vt	vt	vt	vt	vt	vt	r	r	rr	rr	vt	vt
	1921	vt	vt	vt	vt	vt	vt						

All these forms exhibit a fairly uniform periodicity, their development being most pronounced during the warmer months, the maximum generally occurring at the end of summer or in early autumn. In general moderately high temperatures are preferred¹, although it appears that with certain of the species the higher summer-temperatures are distinctly unfavourable². The next most important factor is the concentration of the water, and in the present pond a moderately high concentration between 16 and 22 degrees appears to supply the best conditions as far as this factor is concerned (cf. however *Pediastrum* below). No relation to bright sunshine is apparent.

The aquatic animal life of the pond is another factor of importance influencing the development of these algal species, since, of course, many aquatic animals feed voraciously upon them. In Hawkesley Hall pond animal life was fairly well developed throughout the greater part of the year, although rather more abundant during spring and early summer; and this may have caused the various members of the Protococcales to attain their maximum development rather later in the year than they otherwise would have done.

¹ This agrees with the observations of West (31) who states that the great majority of the Tetrastorine and Chlorococcine Protococcales are late spring and summer forms, often occurring far into the autumn.

² Fritsch and Rich (18) found that *Sphaerocystis Schræteri* was adversely affected by high summer-temperatures in Barton's pond.

Dictyosphaerium Ehrenbergianum (Fig. 10) is a perennial constituent of the algal flora of the pond, its maxima falling in the middle or towards the end of summer (June—July 1918, July—August 1919, August 1920). The most favourable temperature appears to be a monthly mean between 12° and 15° C. (cf. data in Fig. 2), and higher temperatures appear to be rather harmful, as shown by the decline of the species in July 1918, at a time when other conditions seemed favourable. From a comparison with the concentration-curve (in Fig. 3), it is apparent that gradually rising concentrations between 16 and 22 degrees are those most suitable for this species in the present pond, while a decreasing concentration—even between these limits—is distinctly unfavourable. The sudden decline of the species at the end of August 1919 (cf. Fig. 10) was due either to the high concentration of the water (27 degrees) attained in this month, or to the sudden decrease in the concentration during August—September, or again to the high temperature of August, or, more probably, was the result of more than one of these factors.

*Ankistrodesmus falcatus*¹ (cf. above table) is also adapted to moderately high temperatures (a monthly mean between 11° and 14° C.); and again higher temperatures apparently caused a decrease of the species in July 1918. In 1920, probably on account of the favourable temperature-conditions (see Fig. 2), the maximum persisted well into the autumn. The relation towards the concentration of the water is much the same as with the last species considered, the most favourable concentration for growth lying between 16 and 22 degrees, although the species may persist in fair quantity with concentrations outside these limits. A falling concentration does not appear to be so harmful to this species as it was with *Dictyosphaerium*.

Scenedesmus denticulatus var. *linearis* closely followed *Dictyosphaerium* in its occurrence, although it is a decidedly more sensitive form. A monthly mean temperature between 12.5° and 15.5° C. appears to supply the best temperature-conditions for growth; and again higher summer-temperatures were concerned in bringing about a decrease of the species (in August 1918, and perhaps August 1919) after the July maximum, although the concentration of the water probably also played a part. The species increased to a maximum generally when the concentration was rising and between 16 and 21 degrees. The slight lowering of the concentration during June—July 1920 (cf. Fig. 3) apparently did not have any adverse influence on

¹ The vars. *acicularis* and *spirilliformis* of this species showed a periodicity in the pond similar to that of the type, but they were always much less abundant than the latter

this Alga, but in each of the three complete years the decline after the maximum coincided exactly with a marked decrease in the concentration, a coincidence which can hardly be considered as accidental.

Pediastrum Boryanum and *P. tetras* are both favoured by the higher summer temperatures, a monthly mean temperature (of the air) between 13° and 16° C. being requisite for pronounced growth; and in no case did a high temperature ever influence these two species adversely. Little relation is shown by *Pediastrum* towards the concentration of the water. In the case of *P. Boryanum* the increase to the normal maximum may take place either when the concentration is very high (August 1919), or moderately high (June—July 1918 and July—August 1920); while the moderately low concentrations during September—October 1920 apparently affected neither species of this genus adversely, the decline at the end of October 1920 being due to the lowered temperature. Temperature thus appears to be far more important than concentration of the water in determining the development of *Pediastrum*.

In concluding this section it may be noted that these five members of the Protococcales, in regard to the temperatures most favourable for their development, form a sort of series, at one end of which is *Ankistrodesmus*, for which a monthly mean temperature of the air between 11° and 14° C. is most suitable and the higher temperatures unfavourable, while at the other extreme there are the two species of *Pediastrum*, adapted to the highest summer-temperatures, while the two remaining forms are intermediate as regards their behaviour towards temperature.

(To be continued)

A NEW METHOD OF VEGETATIVE MULTIPLICATION IN *CROTALARIA BURHIA*, HAM.

By R. H. DASTUR, M.Sc. AND W. T. SAXTON, M.A.

(With Plate II and 1 figure in the text)

INTRODUCTION

WHILE investigating some ecological problems on an area close to Ahmedabad, the writers made a preliminary investigation of the root structure of typical plants of the formation studied. That of *Crotalaria burhia* presented some peculiarities of sufficient interest to warrant a separate account. This species is perennial, while the other local species of *Crotalaria* are annuals.

EXTERNAL EXAMINATION

The root in the youngest plant¹ we have observed is a straight tap root about 4 ft. long, and in a plant of some years old, the tap root is estimated to be about 16 ft. in length. The actual length dug out on one occasion was 12 ft. but the entire root was not taken out. There are many lateral roots from deeper portions of the main root in younger plants but the development of dormant lateral roots takes place in the upper part also as the plant gets older. In a plant about a year old, the axis is seen to be ribbed, the ribs appearing first in the transition region from root to stem and extending downwards into the root where they gradually disappear. In old plants the ribs are thick and conspicuous (Plate II, fig. 1). They also anastomose with one another as they pass downwards.

A well grown plant reaches two to three feet in height and bears linear leaves $\frac{1}{2}$ in. by $\frac{3}{16}$ in. In a young plant there is only one unbranched shoot. Branches appear in succession all over the upper part of the main shoot. On the lower part close to the base of the main shoot other branches grow from buds, and as the plant gets older they all grow almost to the size of the main shoot and may give rise to secondary branches. The ribs already described end just above the base of the main shoot, extending also into the base of the

¹ Probably less than one year old.

lowest branches and completely surrounding them. The various branches described above we propose to distinguish by the word "normal."

After a long time the main shoot with the upper branches dies back to the point where the ribs surround it, and that portion of the main shoot increases in size owing to the ribs getting thicker and more prominent than before. Both these phenomena occur later in the case of the lower branches also.

Afterwards small buds appear terminating some of the ribs, earlier in the main shoot than in the lower normal branches. These give rise to new branches different in origin (as will appear later) from the "normal" branches and we propose to call them "reproductive" branches. These reproductive branches, which appear ribbed for a short distance from the base, give rise to secondary *reproductive* branches below and secondary *normal* branches above.

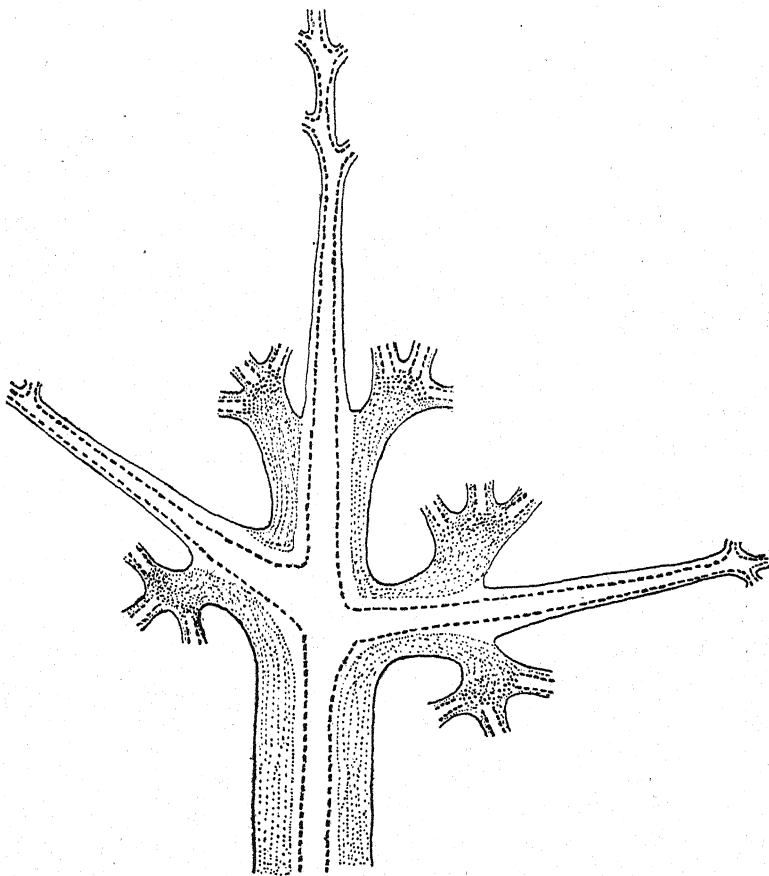
INTERNAL STRUCTURE

A series of transverse sections of the root from the apex to the base was prepared and examined. There is a central cylinder in which secondary thickening takes place at a very early stage, so much so, that it is very difficult to identify the protoxylem in sections prepared only 6 ins. from the apex of the root. The marked feature of the secondary thickening is the great thickness of the phloem. On examining the sections it was clear that the ribbed appearance of the root and portions of the main shoot and branches already described was due to the formation of accessory bundles.

From the sections cut near the apex it was observed that the formation of the cork cambium takes place very early, in the cortex just outside the endodermis. The cells of the cortex are quite typical and differ in form from those of the pericycle in which cork cambium next appears, and the endodermis thus disappears. The cells just below the cork then become meristematic and the first xylem vessel of the accessory bundle appears. Such single xylem vessels appear almost simultaneously at five or six points near the periphery (fig. 2). It seems clear that they are formed in the pericycle. New xylem elements are added to each as well as some phloem, and small bundles are thus produced which project radially (fig. 3). Many such accessory bundles appear in succession, some of them in approximately radial rows. The later formed bundles are always either outside previously formed ones or at fresh points on the circumference (figs. 4 *a* and 4 *b*). Cork formation takes place around the various accessory bundles,

but for a time these remain connected. The cork formation also takes place between the central cylinder¹ and the accessory strands.

The vascular supply of the normal branches consists as usual of branches from the central cylinder of the main shoot. In this way



Text-fig. 1. Diagrammatic longitudinal section to show the way in which the accessory system gives rise to reproductive branches independently of the main central cylinder. The thick broken lines show the normal vascular tissue, the thin dotted lines the accessory bundles.

they are directly connected with the conducting tissues of the main root. The accessory bundles surround the lower part of the main

¹ The term "central cylinder" is here and hereafter used to denote the cylinder of primary vascular bundles, and not, obviously, in the more usual sense of all tissues within the endodermis.

shoot and the normal branches and those regions gain in thickness as the accessory bundles increase in number and grow older. The reproductive branches, which appear later in these lower regions of the main shoot and normal branches, arise directly from accessory bundles and have no connection with the central cylinder of the shoots on which they are borne, nor consequently with the main strands of the root (Text-fig. 1). The same remarks apply to secondary reproductive branches produced on the primary reproductive branches. Thus all these reproductive branches differ in origin from normal branches. But normal secondary branches which are also produced on these reproductive branches are directly connected to the central cylinder of the branches on which they arise. So in old plants, the original vegetative shoot and the normal branches do not exist, and all the shoots are connected only with the accessory bundle system. The central cylinder of the main root dies back from above, after the original shoot and normal branches are dead, and in old plants, a hollow tube of the accessory strands, with decayed matter inside, may be seen extending some distance below the ground. In old plants the accessory strands also separate into groups owing to the formation of cork, but they remain connected below. The groups of accessory strands, as has been seen, terminate in "reproductive" branches, and the latter are therefore carried further apart by the separation of the groups. The accessory system remains attached to the main root below, where the formation of cork has not taken place, and where the accessory bundles decrease in number and become less conspicuous, until, traced downwards, they disappear.

Much light was thrown on many points in this investigation by the preparation of a skeleton of the root, along with the lower portions of the main shoot and branches, by maceration. The skeleton was prepared by allowing it to decay in water for a fortnight and then keeping it in dilute nitric acid for a day. It was quite clear then that the reproductive branches arose directly from the accessory bundles and had no connection with the main central cylinder.

The accessory system in the root at first appeared to be entirely separate from the central cylinder, and it was thus difficult to see how it could function as a conducting system as it did not seem to develop in relation to any absorptive tissue. It was found, however, from sections cut at points where lateral roots originated, that the conducting tissues of the lateral roots, though formed primarily as a branch of the central cylinder, were connected by a few strands with the accessory bundles. The central cylinder becomes functionless

as the main shoot and the normal branches die, and the lateral roots then function as the lateral roots of the accessory system.

Accessory bundles are also found at a higher level in the shoots, both normal and reproductive, but they arise quite independently of the accessory system we have described, and are small regular and inconspicuous externally. These are incorrectly described in a recent paper by Mr Sabnis(1) as anomalous cortical bundles, but as they arise inside the endodermis it is obvious that the word "cortical" is a misnomer.

DISCUSSION

The observations described in this paper make it clear that the whole development of the accessory bundle system is directed to the gradual separation of reproductive branches which will eventually become established as separate plants. So far as we are aware, such a provision for vegetative reproduction is markedly different from anything previously described. The plant is a prominent member of a psammophytic association in which a likelihood of partial burying in the sand clearly exists, and though we have not actually seen the process taking place, our observations point to this conclusion, since much diversity exists in the depth at which the transition region is met with in plants of the same age. Such a state of affairs will obviously accelerate the separation of branches initiated by the process we have described. We thus see that when the main axis of the plant perishes, a circle of branches separated to below the ground level is already established, and it cannot be doubted that the separation must eventually become complete.

The family Leguminosae is well known to be remarkable for the frequency with which abnormal or unusual vascular structure is met with, but none of the accounts of such structures indicate, so far as we are acquainted with them, that they are connected with any specialised method of vegetative reproduction, nor do we think, regarded purely as anatomical peculiarities, that the structures hitherto recorded are quite comparable with those here described. Further reference to literature would therefore appear superfluous.

It is worthy of notice that though the plant is seen flowering during the major part of the year it seldom seems to develop seeds. This season, however (November, 1920), the mature pods have been collected in large numbers, a phenomenon which we attribute to the almost complete failure of the latter half of the monsoon.

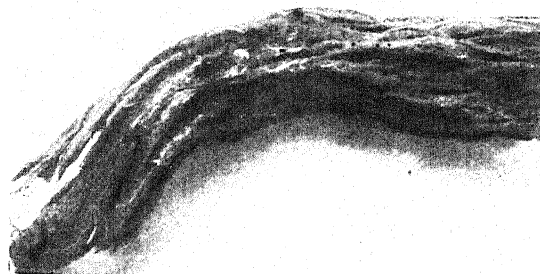


Fig. 1

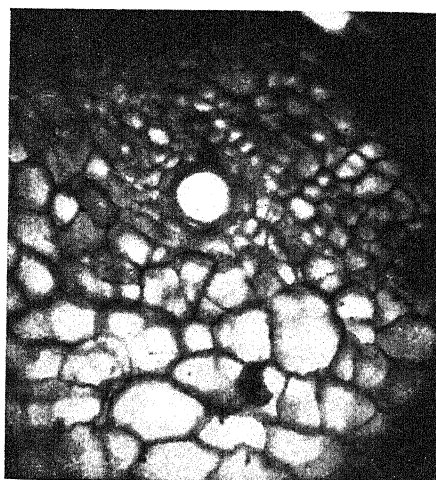


Fig. 2

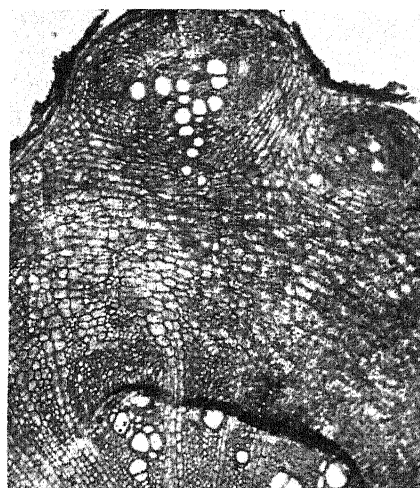
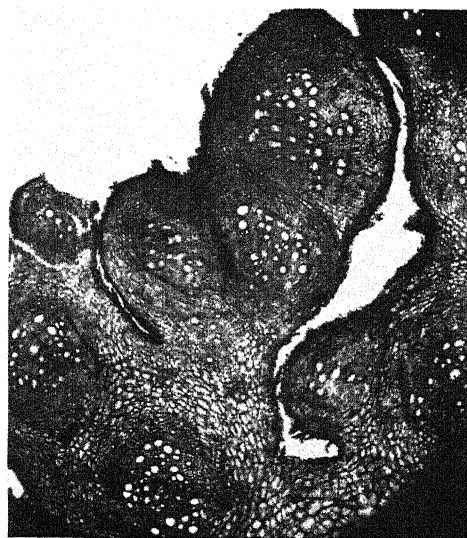
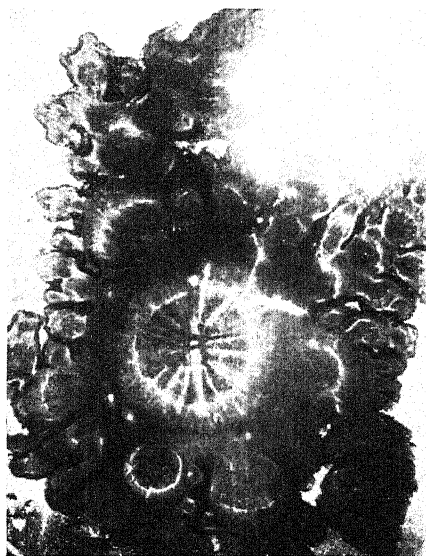
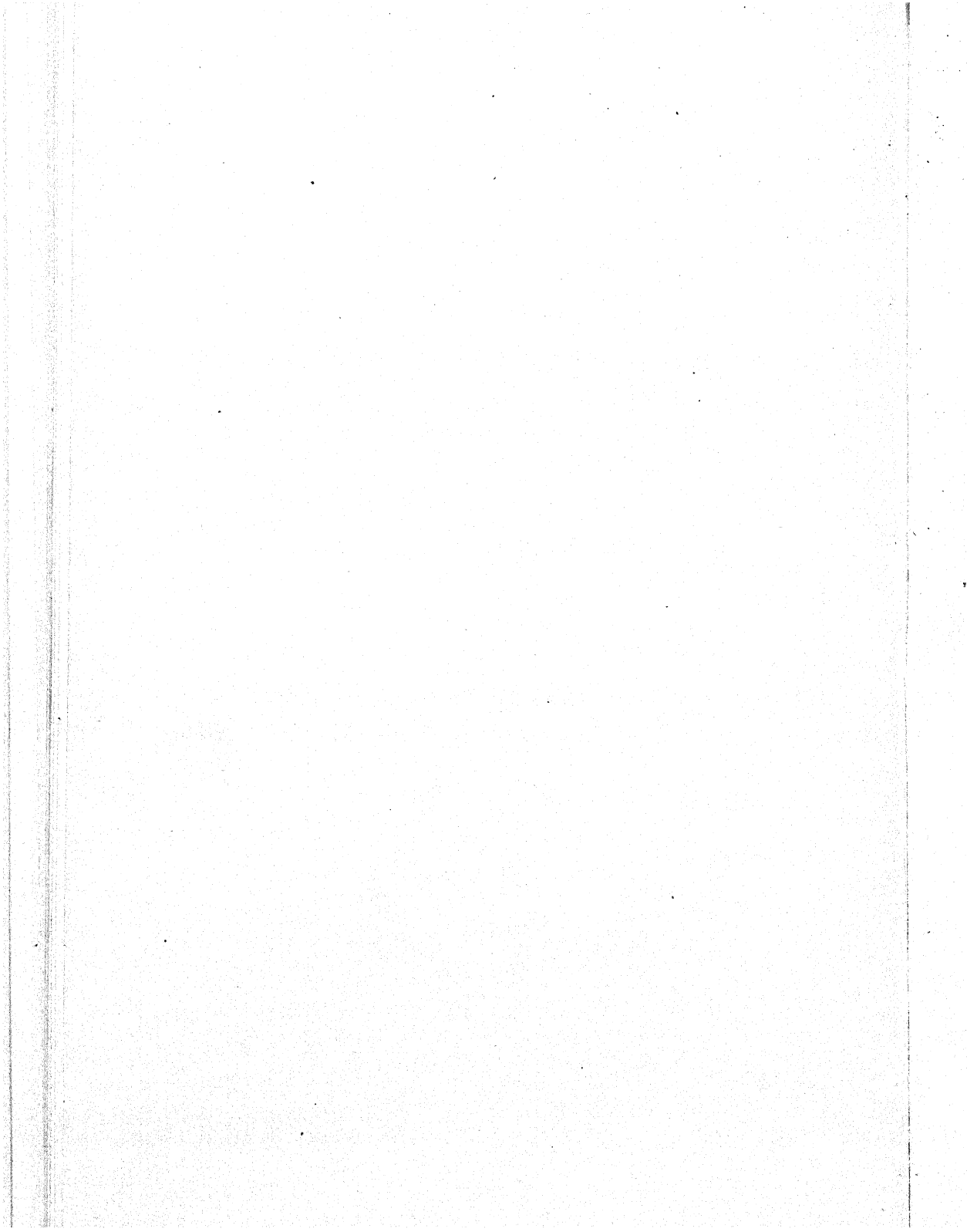


Fig. 3





ACKNOWLEDGMENT

We desire to record our indebtedness to Mr A. G. Tansley, F.R.S., for some helpful suggestions made to one of us during the progress of this investigation.

NOTE. This investigation was carried out in the Botanical Laboratory of the Madhavlal Rauchhodlal Science Institute, Ahmedabad.

LITERATURE CITED

- (1) SABNIS, T. S. The physiological anatomy of the plants of the Indian Desert. *Journ. Ind. Bot.*, 1, p. 197 and Fig. 101. March, 1920.

EXPLANATION OF PLATE II

Fig. 1. A piece of an old root. $\frac{5}{8}$ natural size.

Fig. 2. Transverse section of a young root, showing origin of accessory bundles. $\times 160$.

Fig. 3. Older stage. $\times 33$.

Fig. 4 *a*. A thick section, almost complete, of a late stage. $\times 3\frac{1}{2}$.

Fig. 4 *b*. Part of a similar, but thinner, section, showing detail. $\times 25$.

A NEW SPECIES OF *CÆLASTRUM*

By FLORENCE RICH, M.A.

(From the Botanical Department, East London College)

IN making collections of algæ from various pieces of water in Leicestershire a sample was gathered on April 26, 1915, from a very narrow ditch (choked with phanerogamic vegetation and much overhung with ivy, brambles, etc.) that bounds the garden of the large house known as Leicester Frith, situated on Boulder Clay overlying Keuper Marl, on the Groby Road, near Leicester. When this was examined it was found to contain three species of *Spirogyra*, of which *S. quadrata* (Hass.) Petit was the commonest, *Vaucheria sessilis* (Vauch.) D.C., many diatoms, and numerous small colonies of cells which proved to belong to an interesting and apparently undescribed species of *Cælastrum*. Repeated attempts to obtain fresh supplies of material were unfortunately without success. In the original sample, however, there is a considerable amount of material available, and the organism can be observed in many different stages (of which a few have been figured).

The cœnobium of the new species of *Cælastrum* consists of 2, 4, 8 or more, rounded cells; perhaps 8 is the commonest number. Single cells are frequent, and apart from the typical *Cælastrum* grouping irregular colonies occur (Fig. xv). The cells vary in size from 11μ to 14μ in diameter.

The cœnobia are sometimes surrounded by a wide—or narrow—mucous investment with a well-defined boundary, but more often than not this investment is wanting.

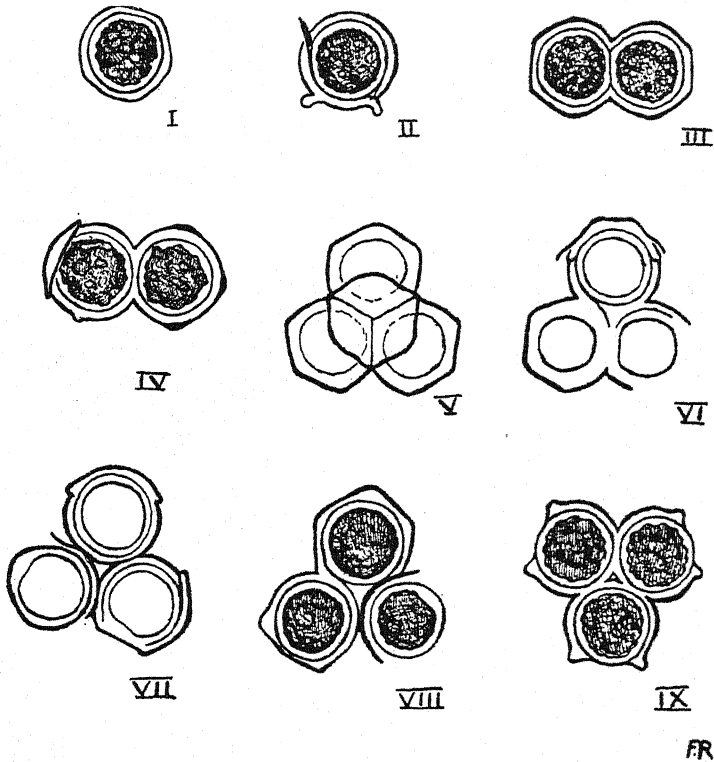
The cell-contents (in the preserved material) are light bluish green in colour, the smaller (? younger) cells showing a more yellowish tinge. The chloroplast has not been completely deciphered, but there *appears* to be one in the form of a convoluted plate. There seems to be one pyrenoid in each cell. The contents show the presence of plentiful starch.

The cell-wall is laminated and is evidently rather mucilaginous as it is penetrated by methyl blue.

Isolated cells are, roughly speaking, spherical in shape; when there are groups of two or more the cells, though still frequently

round, are very often angular. The cells that show this "rounded angularity" are quite symmetrical, triangular in optical section, with a suggestion of three other intervening angles, thus appearing faintly hexagonal (Fig. v)—like the transverse section of the ovary of *Viola*.

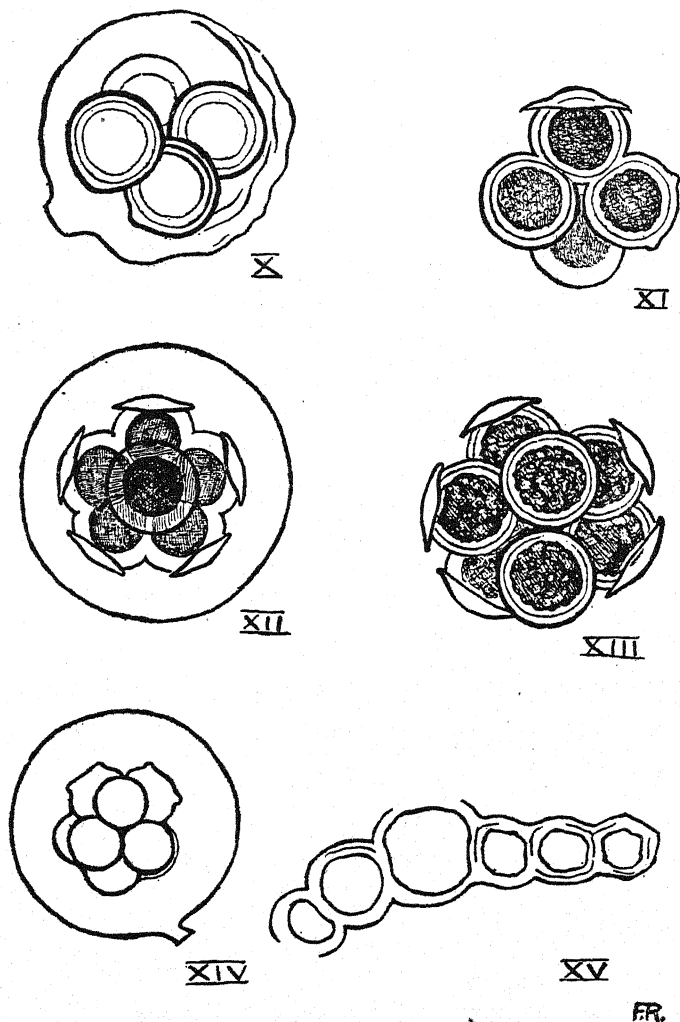
When the cells are spherical the spaces between them are very small, much smaller than in other species of *Cœlastrum* (even *C. microporum* Naeg.), and these spaces are triangular in form (Figs. VIII and IX).



Figures of *Cœlastrum schizodermaticum* (magnified about 900 times).

- I. Single cell showing slight departure from spherical shape.
- II. Single cell showing splitting membranes.
- III. A two-celled individual showing angularity of cells.
- IV. Thickening at angles, and formation of one cap.
- V. Colony of four cells.
- VI. Colony of four cells, one cap splitting off.
- VII. Showing splitting away of outer membrane.
- VIII. The same.
- IX. Colony of four cells all having lost caps.

With regard to the grouping in the eight-celled families it is found that sometimes there are two superimposed tiers of four cells each,



Figures of *Caelastrum schizodermaticum* (magnified about 900 times).

- X. Four-celled colony showing connection of mucous investment with caps.
 XI. A four-celled colony with a very clear and conspicuous cap on one cell.
 XII and XIII. Mature cenobia with, and without, investment.
 XIV. Investment showing stalk-like protuberance.
 XV. An irregular colony.

the members of one tier alternating with those of the other; sometimes, however, the *appearance* is of five cells in one plane with one cell above and two cells below (Fig. XII).

The cells of the *cœnobia* cohere by means of broad flattened surfaces and there are no connecting rods or tubercles.

The peculiarity of this organism is the splitting off of little cap-like structures from the free surfaces of the cells. First of all minute pads appear, about one-fifth or one-sixth of the circumference apart (Fig. IV); then fission of the outer layer of the cell-wall seems to occur along a line connecting at least two of these pads, and a little circular or four-cornered cap is detached (Figs. XI, XII and XIII). An eight-celled colony with the little caps well defined presents a very characteristic appearance. Sometimes fission of the wall occurs along the surface attached to other cells; in this case, of course, the cell in question ultimately breaks away and the cap-shaped structure is left adhering to the residue of the main colony like a little saucer (Figs. VII and VIII).

The small pads may correspond to the special short truncate processes or warts that unite the cells in other species of *Cœlastrum*, e.g. *C. Morus* W. and G. S. West, *C. scabrum* Reinsch, *C. cambricum* Archer, and *C. reticulatum* Danz. It may be the case that the cell-wall is becoming more mucilaginous at these spots, and that this causes splitting off of the caps. It is possible that the caps themselves ultimately become mucilaginous and merge into the mucilaginous envelope, as is indicated in Fig. X where folds in the investment seem to pass over into the little caps.

With regard to multiplication, the formation of auto-colonies by the cells of an entire colony has not been observed. Cases, however, like that shown in Fig. XIV would seem to indicate the possibility of formation of daughter colonies by single cells which may have been produced by disintegration of adult *cœnobia*, the daughter-colony being at first enclosed within the mucilaginous wall of the parent cell. The stalk-like protuberance of the investment in Fig. XIV may have resulted from the gelatinisation of one of the incipient caps.

The following Latin diagnosis is appended:

Cœlastrum schizodermaticum n.sp.

C. coenobiis globosis, tegumento mucoso interdum praeditis, e cellulis sphaericis vel subangularibus confertis, vulgo 8, constantibus, faciebus deplanatis cohaerentibus, membrana lamellata; lacunis inter

cellulas minutissimis triangularibus; lamella externa membranae saepe in forma mitrae circularis vel quadrangularis soluta est; chromatophora singula (?) in quaque cellula cum pyrenoide; multipliciter non satis cognita.

Diam. coenob., ca. 35μ ; diam. cell., 10-14 μ .

My thanks are due to Prof. F. E. Fritsch for the generous help he has given me in studying this little organism.

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LEICESTER.

NOTES ON AN EASY METHOD OF CAMERA LUCIDA DRAWING

By JAMES SMALL

(With 2 figures in the text)

A COMBINATION of Baker's "Reflex Drawing Apparatus" with an Ediswan "Pointolite Lamp" has proved so satisfactory in working that these notes have been written to make known the ease and speed with which a clear *camera lucida* drawing can be made by this method.

The Reflex Drawing Apparatus¹ is a small right-angled prism with the 45° side silvered (the whole protected by metal fittings), which is affixed by means of a small screw upon the top of any ordinary eyepiece. It possesses several advantages over the larger mirrors in common use. Firstly, it is small, convenient to handle, easily adjusted, and when affixed to the eyepiece it can be readily turned up so that the field may be viewed through the mirror from above. Secondly, the mirror surface is protected by glass which is exposed only to a small extent; this largely obviates the numerous cleanings necessary with the larger mirrors, and the deterioration of the surface which is a consequence. Thirdly, since the rays pass into

¹ Sold by C. Baker, 244, High Holborn, London. Price, 17s. 6d.

and out of the glass at right angles to the surface, double reflections are avoided and the reflecting apparatus becomes as efficient as a simple metallic mirror.

The "Pointolite" outfit¹ is becoming so well known, and is, indeed, so rapidly becoming one of the recognised pieces of equipment for microscopic work, that description is unnecessary. Special stands and holders for the lamp are supplied, but in the present case the lamp-holder was attached to a heavy base with a universal ball and socket joint which happened to be available. For drawing purposes the lamp is arranged in the vertical position and covered with an oblong

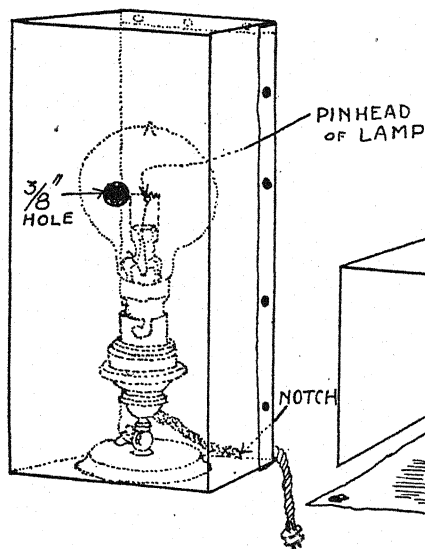


Fig. 1

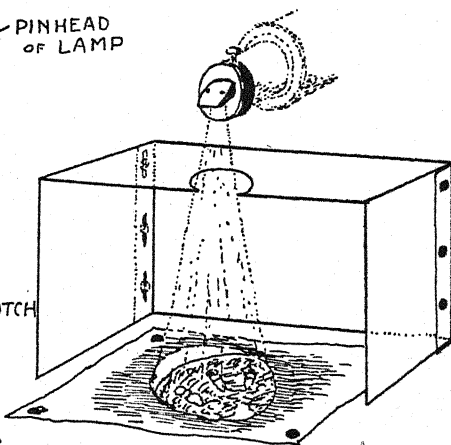


Fig. 2

case of cardboard. This case (Fig. 1) has a small notch in the base, through which the flexible wire is passed, allowing the base of the case to rest flat upon the bench. In one side of this cardboard cover and exactly on a level with the small "pinhead," which forms the source of light, a round hole not more than three-eighths of an inch in diameter is cut.

The substage mirror is removed from the microscope, or swung to one side, and the microscope is tilted into the horizontal position. The height of the tube in this particular case is slightly less than that

¹ The Standard 100 candle power lamp has been used (cost 24s.) with the Standard Universal Resistance Box and fittings (cost £5, less 33½ %), as supplied by the Edison Swan Electric Co., Ponders End, Middlesex.

of the hole, and the instrument is raised by flat wooden blocks until the optical axis is on a level with the centre of the hole and with the glowing pinhead of the lamp. It is pushed as close up to the hole in the cardboard casing as the stand will allow. The section to be drawn is then inserted, and the chosen field illuminated as evenly as possible and as brightly as is compatible with clear definition of details. The Reflex Drawing Apparatus is now screwed on (the appearance of the field can be checked by turning the mirror upwards) and then the mirror is turned so that a bright, clear picture showing details and colouring as in the direct view is thrown upon paper placed flat upon the bench.

The brightness of the image upon the paper varies, of course, with the amount of light from other sources. The stray beams from the hole in the cardboard casing have little effect, if the apparatus is properly fixed up. The image is faint in full daylight, but in twilight or when dark blinds of the now usual type are pulled over the windows the image is quite bright. Final adjustments of brightness can be made easily by using (1) a half cover of cardboard (Fig. 2) which shades the image from all extraneous light¹ except from the observer's direction and his shadow acts very efficiently from that side; (2) an ordinary night-light which can be moved about until lines drawn upon the paper are visible while the image still remains clear. Nothing more remains to be done now, except to draw lines in pencil or in ink around the outlines of cells and to fill in as many other details as are required in the same way; the actual process of drawing becomes easier and more rapid than tracing with transparent paper.

The advantages of this method are—firstly, the ease with which the adjustments can be made when the apparatus *has been* in working order; secondly, the brightness and clearness and flatness of the image which make it possible to work in daytime without a dark-room; thirdly, the ease and comfort with which a *camera lucida* drawing can be made when one has the paper flat upon the bench and when both image and paper are viewed directly, and not one or other as a reflection; fourthly, the comparatively low price of the apparatus, especially when it is considered that apart from the reflex mirror the rest of the apparatus is useful for so many other illuminating purposes. The total cost is, in fact, less than that of many of the usual *camerae lucidae*.

¹ These cardboard cases can be made easily by ruling heavily with a blunt point the lines for folding, and then fixing the edges which overlap with split-pin paper fasteners or drawing-pins.

One of the great difficulties in making drawings of consecutive portions of a large section is that the end of one portion only fits exactly to the beginning of the next portion when the plane of the drawing paper has been very accurately adjusted in the same plane as the object or the image. Using this method, one can move the section by hand or on the mechanical stage and leave a part of the section already drawn still in the field. This part can be fitted easily on to the drawing by moving the latter in the direction followed by the moved image. The outlines of both are clear and can be superimposed quite accurately and easily, and then the drawing of the new portion can proceed. These adjustments only involve the turning of the mirror into the correct plane and this can be done rapidly, because it is only necessary to get a circle of light and not an ellipse. If two diameters of the illuminated zone at right angles to each other are equal the adjustment of the mirror is accurate.

As examples of diagrams drawn in pencil or directly in ink by this method in from two to ten minutes readers are referred to my *Textbook of Botany* (Figs. 148, 150, 270 *a*, 395, 399, 400), while for detailed drawings occupying never more than one hour, and sometimes only fifteen minutes for the pencilling, they may note the Figures 149, 151, 153, 154, 157, 268, 270 *b*, 274, 308 and 396 in the same volume.

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AN UNUSUAL PLANT OF *CHEIRANTHUS* *CHEIRI* L.

By ETHEL M. POULTON, M.Sc.

University of Birmingham

(With 16 figures in the text)

IN May, 1921, the writer observed a curious plant of *Cheiranthus cheiri* growing in the garden of Merion Lodge, Hagley, Worcestershire. On examination, the plant was found to exhibit interesting deviations from the usual structure.

Though the inflorescence was of the normal racemose type the general appearance of the plant was quite unlike an ordinary wall-flower. This was partly due to the inconspicuous nature of the flowers. The petals were greenish-yellow, simple in shape, showing no differentiation into limb and claw, somewhat concave, and much reduced in size. Indeed, they were smaller than the sepals and therefore almost enclosed by the calyx (Figs. 1, 1 a). The sepals were of the typical shape, size and number ($2 + 2$; 2 saccate), but were inclined to be petaloid. Their margins and apices were usually yellowish. The flowers gave the impression of unopened flower-buds rather than mature specimens. Sepals and petals had dropped from the oldest flowers, but no fruits appeared to be forming. This circumstance may probably be correlated with the absence of an attractive corolla. Self-pollination could not occur for reasons which follow.

The most striking feature of the flowers was the entire absence of functional stamens and their replacement by carpels in various stages of development, a stigma and minute ovules being formed in many cases. The characteristic number (6) was everywhere preserved, and often the usual arrangement ($2 + 4$) could be observed. Another curious feature was the tendency of the rudimentary carpels to fuse with the central gynæcium forming a composite structure (Figs. 2, 3, 6, 11, 13)¹. This fusion was partial or complete, both as regards individual "staminate carpels" and the whorl as a whole. Where such fusion occurred the seam was usually well marked. The car-

¹ In Figs. 2, 3, 6, 10, 11, 12, the calyx and corolla have been removed.

pellary nature of these structures was shown in one or more of the following ways:

- (a) An incurving of the margins, along which minute ovules were formed (Figs. 7, 9);
- (b) the formation of a stigmatic surface, often swollen but always covered with minute excrescences as in the typical stigma (Figs. 7, 8, 11, sg);
- (c) the outer surface was pale green and covered with a fine down as in normal carpels.

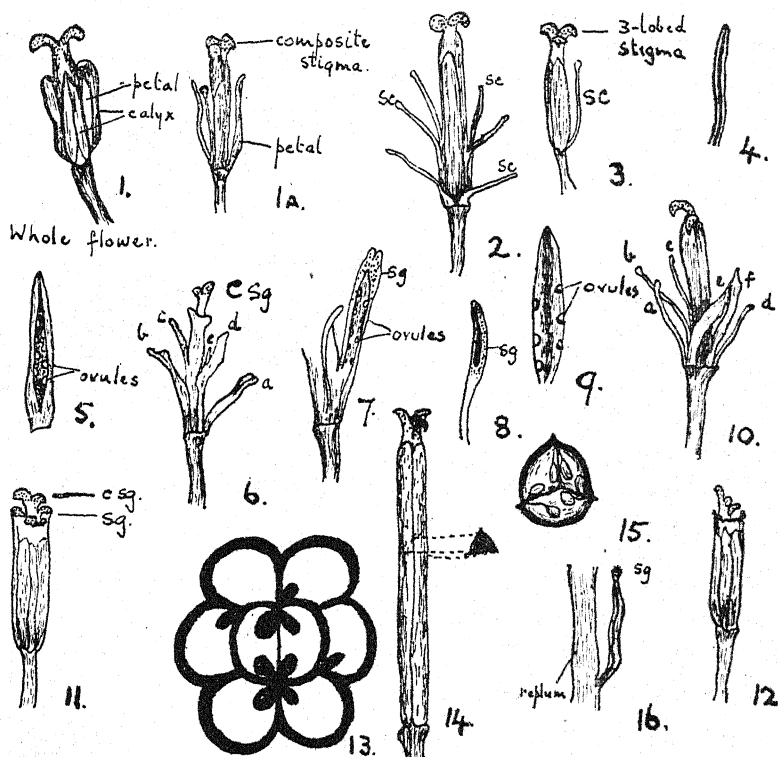


Fig. 2 shows six "staminate carpels" (sc) joined to the normal central gynæcium only at the base, the upper parts being free. The central gynæcium is of the ordinary wallflower type. In Fig. 3 is seen a 3-lobed stigma in the centre of the flower, one free "staminate carpel" (sc), the others having fused with the wall of the central gynæcium. Fig. 4 shows a petal with the edges curling towards each other, while Fig. 5 represents a "staminate carpel" from the same

flower, with the margins closing over and forming small ovules. The remaining parts of the same flower (after the removal of calyx and corolla) are seen in Fig. 6. The central stigma, *csg*, is 2-lobed, *a-e* are "staminate carpels" all of which are joined at the base; *a* and *b* tend to curl over at the tip, and produce minute ovules in various stages of development. Each also has a distinct stigmatic surface. Fig. 7 is an enlarged view of *b*, showing the ovules, and the stigmatic surface *sg*.

In Figs. 8 and 9 are seen "staminate carpels" from other flowers.

Fig. 10 represents four barren "staminate carpels" (*i.e.* without ovules) *a-d*, and two better developed ones *e, f*, which unite with each other and with the base of the main gynæcium. These (*e, f*) had rudimentary ovules on their margins. In Fig. 11 the union is almost complete, a solid column being formed of the central gynæcium and the six "staminate carpels" the stigmas (*sg*) being separately recognisable. The normal 2-lobed stigma, *csg*, is seen projecting from the surrounding stigmatic surfaces (*sg*). Fig. 12 shows the same flower rotated through 90°.

A cross-section of this composite gynæcium is shown somewhat diagrammatically in Fig. 13. The main gynæcium is seen in the centre and is of typical structure. The six "staminate carpels" have completely fused with it. The plane of the section has passed through some of the ovules formed by these.

Thus, the suppression of maleness, as regards its ordinary manifestation is complete throughout the whole plant, and the tendency to femaleness is strongly marked. It is possible that even the corolla shows this tendency in the curling over of the edges of some of the petals (Fig. 4). Very significant is the preservation of the characteristic number and arrangement of the stamens, as though the primordia of these had been formed as usual. It is difficult to resist speculation. Perhaps one might hazard the suggestion that an absence of the factor (or factors) making for maleness, or a preponderance of that (or those) associated with femaleness, might account for these curious divergencies from the familiar structure.

Worsdell(1) describes a similar abnormal flower of *Cheiranthus cheiri* and states that it is an example of "carpellody" which "is a frequent phenomenon revealing to us the fact that the stamens and carpels are very closely allied organs, and the facility with which the one may change into the other, doubtless due to the fact that both are derived from a common ancestor, the asexual sporophyll which exists to-day in some of the more primitive types of plants such as ferns, horse-tails, and some lycopods." (2)

Masters⁽³⁾ states that de Candolle mentions such a plant in his *Prodromus* as a distinct variety under the name of *gynantherus*. A description of a similar flower by Brongniart⁽⁴⁾ is quoted. This, however, differs in some details from the case described above. The "staminate carpels" were in some cases fused into two lateral bundles of three each, and in many instances the two outer stamens were entirely suppressed. He states that the two shorter stamens undergo change into carpels later than the longer ones, a point which the above specimen did not illustrate.

ADDENDUM

Figs. 14-16 are taken from a different wallflower plant, growing in the vicinity of the one described above. They represent the gynæcium of the only abnormal flower in the whole inflorescence. The peculiarity here is the 3-carpellary ovary with a 3-fid stigma (Fig. 14). In the section, three partition walls are seen dividing the ovary into three chambers. At the base of the ovary, arising from the septum was an out-growth resembling a "staminate carpel" of the above specimen (Fig. 16). The edges were folded inwards, the outside was downy, and the tip was covered with the minute excrescences characteristic of the typical stigma.

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- (1) *Principles of Plant Teratology* (Ray Society), 2, p. 184.
- (2) *Op. cit.*, p. 182.
- (3) *Vegetable Teratology* (Ray Society), pp. 305-6.
- (4) *Bull. Soc. Bot. France*, 8, p. 453.

THE THEORY OF GEOTROPIC RESPONSE

By V. H. BLACKMAN

I HAVE read with some surprise Prof. Small's reply in this Journal (20, pp. 73-81, 1921) to my criticism of his theory of geoperception. I understood that the cell particles which "creamed" were the ultramicroscopic disperse phase of the protein sol of the protoplasm, a view which I find was taken generally by botanists. Prof. Small now tells us that we are all mistaken in our interpretation and that the theory applies to the much larger visible particles of the granular protoplasm. The basis of this misunderstanding appears to be the author's somewhat peculiar application of the term "disperse phase." On his assumption protoplasm consists of an "emulsion of protein or protein-lipoid particles in a colloidal protein medium." The system postulated has thus two disperse phases; the author, however, applies the term "disperse phase" solely to the particles, and neglects altogether the disperse phase of the protein sol, to which on the ordinary colloidal theory the special properties of protoplasm are due. It is, therefore, not surprising that confusion has occurred, especially as references to iso-electric points naturally lead one to believe that protein sols are being considered. His scientific colleagues can, I think, with some justice complain of the vagueness of outline and the lack of detail in the picture which Prof. Small presented to them. Apart from the difficulty of interpretation just mentioned, such important points as the size of the particles which were to react, the rate at which they were to move, and the extent to which redistribution would take place, were all left quite untouched although they were fundamental to the theory.

The theory has now assumed such a different complexion that it would seem unnecessary to refer to Prof. Small's misunderstanding of some of my criticisms. What is now required is direct evidence of "creaming"¹. There might be some excuse for the publication without such evidence of a theory based on the movement of ultra-

¹ The rate of movement of the large particles cannot be deduced directly from Stokes's well-known equation for, *ex hypothesi*, the particles are producing a difference of potential. They are, therefore, doing work and their movement will thus be retarded, and possibly markedly so.

microscopic particles, but if the theory was from the first one which included the movement of *visible* cell granules an attempt to obtain direct evidence before putting forward the theory would seem more in accordance with the accepted canons of scientific procedure. The examination of meristematic cells with the microscope horizontal should soon supply proof as to whether the cell granules do, or do not, markedly "cream"; and the use of an electric field should accelerate their movement and also decide as to the nature of their charge. Until evidence of movement is available it would seem useless to discuss further the many difficulties which are still implicit in the theory.

THE HYDRION THEORY OF GEOTROPISM

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WITH regard to Professor Small's Hydrion differentiation theory of geotropic response, surely there is a difficulty in the electrical assumptions which has so far escaped notice.

Let us grant for the moment that the creaming process in which various particles rise through the effects of gravity might lead to the production of potential differences. It is still not clear how any current would result even so. For let us consider, for simplicity, one cell in the root, instead of a series of cells. In this cell, we will suppose that there are free positively charged particles, which tend to rise under gravity. This they will do, then, until the forces on them due to gravity are balanced by the electrostatic attractions between them and the relatively negative remainder of the cell. At this point equilibrium will be reached, and there can be no current unless there is a return path for discharge. To suppose that a current will be produced in the reverse direction down the cell is equivalent to supposing that when a Voltaic cell is set up with terminals unconnected, the production of positive and negative poles will cause a reverse current to flow from positive pole to negative *in the cell*!

Professor Small says that Bose has found in stems and roots currents of the required kind. Now it is true that when Bose connects

through a galvanometer two points in a tissue at different potentials, he finds that a current flows round the circuit, but this does not show that the same current was flowing before the circuit was made. Moreover, when the current does flow, since it flows from positive to negative in the wire, it must flow from negative to positive in the tissue, just as in the Voltaic cell, when on closed circuit, the current in the cell flows from negative to positive pole, being forced up against the potential gradient by the internal forces of the cell. Thus in the tissue, when a current is artificially produced, it will flow in the opposite direction to that required by Professor Small. This point of view is in agreement with that of animal physiology, and also of Bose in his *Comparative electro-physiology*, though it is true that in earlier writings (cf. *Plant response*, p. 33) he found some objection to it. But it is of no use to go further into the question of direction of current, until it is made clear what bearing the question has upon Professor Small's theory. On this point we have only his brief statement that a downward flowing current in the root will produce greater effect at the lower side "on account of the resistance in the circuit." (This Journal, 19, No. 3, p. 53.) As I suspect that others besides myself have been puzzled by this statement, may I ask for further explanation?

But the chief source of difficulties is surely the practice of speaking of "currents" in tissues when the evidence only shows that there are potential differences. There appears indeed to be no direct test for currents in tissues in a state of nature, though it is evident that such must occur, by local short-circuiting through cell walls or other paths.

Such currents must, in fact, occur if the two points at different potential are connected also by tracts of conducting tissue other than those along which the forces are acting which produce that potential difference: but then the resulting currents will be circular, flowing in one direction along the one path and in the other direction along the other.